THE RIBOFLAVIN CONTENT OF MEATS[1]

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THREE FIGURES

(Received for publication April 18, 1938)

Since the demonstration that riboflavin is one of the components of the vitamin B complex (György, Kuhn and Wagner-Jauregg, '34 a) interest has been stimulated in the distribution of this substance in animal tissues. Determinations of the 'vitamin G' content of such tissues have been reported (Aykroyd and Roscoe, '29; Hoagland and Snider, '30; Day, '31; Roscoe, '33; György et al., '34; Christensen et al., '36; Kellogg, '36), but one cannot be certain that all of these determinations measured riboflavin.

In general, two methods have been developed for the quantitative estimation of riboflavin. The first of these consists of extracting the material with an organic solvent and comparing the depth of color of the extract with a standard solution of pure riboflavin or some similarly colored substance previously standardized against riboflavin. von Euler and Adler ('34) and Charite and Khaustov ('35) have utilized this technic as a means of measuring the flavin content of animal tissues.

The second general method consists of estimating the amount of flavin in a substance from the growth induced in standard test animals. Since this would measure the content

[1] Research paper no. 516, journal series, University of Arkansas. Aided by a grant from the Committee on National Live Stock and Meat Board Fellowships of the National Research Council.

of biologically active flavin, it would appear to be more applicable to nutritional studies. This method has been used by György ('35) and Carlsson and Sherman ('38) to study natural products of animal origin. Several independent investigators have reported that the 'vitamin G' as measured by the Bourquin-Sherman method ('31) is flavin (Booher, Blodgett and Page, '34; Bisbey and Sherman, '35; Copping, '36; Levine and Remington, '37).

EXPERIMENTAL METHOD

Biological assays have been made of the riboflavin content of beef brisket, lamb chops, bacon, cured and fresh ham and pork liver. The flavin-deficient diet and experimental technic have been adequately described in detail (Day and Darby, '36, '36 a; Day, Darby and Langston, '37). Two casein preparations were used in the experimental diets. They were 'casein, edible, muriatic,'[2] extracted with 60% ethyl alcohol in this laboratory according to the method of Sherman and Spohn ('23) and a 'vitamin-free' casein.[3] These two preparations have been shown to be essentially flavin-free by biological tests and by examination of eluates from them under ultraviolet light (Day and Darby, '37).

The fresh meats were purchased twice a week from local retail markets, and were stored in a refrigerator until used. The uncooked meat supplements to the flavin-deficient diet were weighed out on an analytical balance and fed at once in porcelain ointment pots. The meats were consumed immediately and completely by all the animals except those receiving the largest supplements of bacon. Each meat was fed at three levels to groups of rats under properly controlled conditions. The amounts of supplements indicated in table 1 were fed six times a week, except the pork liver. This was given in twice the indicated quantities three times a week. Only the lean portions of the ham, lamb and beef brisket were fed.

[2] Manufactured by the Casein Company of America, 350 Madison Avenue, New York.

[3] 'Labco' brand, manufactured by the Casein Company of America.

TABLE 1

Data regarding growth and incidence of cataract in rats receiving a riboflavin-deficient diet supplemented with various amounts of meats, and the amount of riboflavin contained in these meats as computed from growth data

SUPPLEMENT [1]	NUMBER OF RATS	NUMBER SHOWING CATARACT	AVERAGE GAIN [2]	RIBOFLAVIN	
			gm.	*units per gram* [3]	*micrograms per gram* [4]
Controls [5]	5	3	—6.2		
Beef brisket, 1.0 gm.	4	0	12.2		
Beef brisket, 2.0 gm.	5	0	43.0	0.74	1.9
Beef brisket, 4.0 gm.	5	0	78.6		
Controls	8	5	—4.9		
Lamb chops, 0.5 gm.	10	2	10.6		
Lamb chops, 1.0 gm.	10	1	27.1	1.1	2.8
Lamb chops, 2.0 gm.	9	0	56.9		
Controls	9	6	—2.8		
Bacon, 1.0 gm.[6]	9	4	5.6		
Bacon, 2.0 gm.[6]	7	0	7.7	0.36	0.9
Bacon, 4.0 gm.[6]	2	0	14.5		
Controls	10	5	—2.5		
Ham, cured, 1.0 gm.	10	3	14.8		
Ham, cured, 2.0 gm.	10	0	39.6	0.81	2.0
Ham, cured, 4.0 gm.	10	0	93.6		
Controls	10	5	—4.9		
Ham, fresh, 0.5 gm.	10	4	15.7		
Ham, fresh, 1.0 gm.	10	0	27.0	1.2	3.0
Ham, fresh, 2.0 gm.	9	0	63.5		
Controls	8	4	—6.1		
Pork liver, 0.05 gm.	10	3	7.6		
Pork liver, 0.1 gm.	10	0	22.4	9.2	23.0
Pork liver, 0.2 gm.	9	0	57.9		

[1] The amount of fresh meat fed six times a week.

[2] Gain in the 8 weeks during which the rats received meat supplements.

[3] One unit is the amount of riboflavin (vitamin G) required to induce a mean growth of 24 gm. in 8 weeks, when fed six times a week.

[4] Expressed in terms of the biologically equivalent amount of crystalline riboflavin.

[5] There was a total of forty-eight control rats used in the series. Of these, twenty-five (52%) developed cataract during the 10-week period, but thirty-two (67%) exhibited cataract before death.

[6] The data on animals receiving bacon are for an assay period of 4 weeks.

It was impossible to eliminate entirely the fat and connective tissue from the samples of brisket to be fed. The bacon fed was sliced, rindless and of average fatness. The samples of bacon weighed out contained lean and fat in approximately the same ratio as they were found in the product as purchased. The high fat content of the bacon appeared to injure

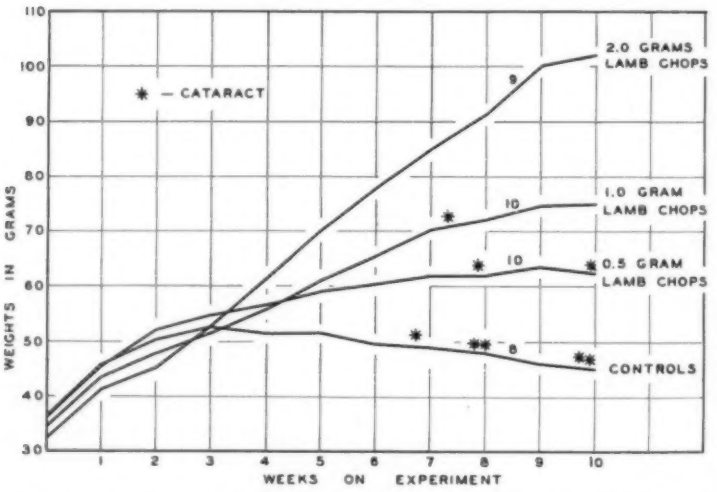

Fig. 1 Average growth curves of groups of rats that received a flavin-deficient diet supplemented with fresh lamb. The rats received only the flavin-deficient diet for the first 2 weeks, and thereafter each animal received the amount of lamb indicated at the end of the curve, six times a week. The numbers on the curves indicate the number of rats, and the asterisks mark the time of appearance of cataract in individual animals.

the animals, and many of the rats receiving bacon died before the end of the experiment. Consequently, the bacon data have been presented for a 4-week period.

RESULTS

Figures 1 and 2 give mean weight curves of groups of rats which received various amounts of fresh lamb and pork liver. The rats were given only the riboflavin-deficient diet for the

first 2 weeks, after which they received the supplements of fresh meat six times a week that are indicated on the curves. Similar data were obtained for the other meats studied, but to conserve space the essential facts are presented briefly in table 1. The mean weights of the groups of rats, when they were started on the experimental diet at 21 days of age, were between 32.6 and 42.3 gm. When the feeding of meats was

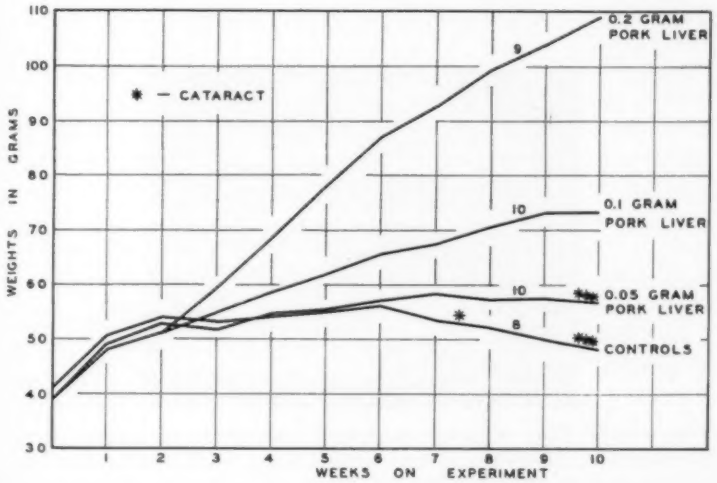

Fig. 2 Growth curves of groups of rats that received a flavin-deficient diet supplemented with fresh uncooked pork liver. The rats received only the deficient diet for the first 2 weeks, and thereafter each animal received the amount of liver indicated at the end of the curve, six times a week. The numbers on the curves indicate the number of rats, and the asterisks mark the time of appearance of cataract in individual animals.

started (2 weeks later) the mean weights of the groups were between 43.6 and 54.0 gm. The animals were fed the meat supplements for a period of 8 weeks and the average net gain for each group is given in the table.

Published experiments (Day, Darby and Langston, '37) have shown that our method of 'vitamin G' assay measures riboflavin, at least when the growth is 10 gm. weekly or less.

The content of riboflavin in the meats studied has been computed from growth data and presented in two ways (table 1). One column gives the number of units of riboflavin per gram of meat. We have employed this unit to mean the amount of riboflavin required, when fed six times a week to depleted animals, to promote an average net gain of 24 gm. during the 8 weeks that the rats were fed the supplements. The meats assayed were found to contain the following amounts of riboflavin (vitamin G): beef brisket, 0.74; lamb chops, 1.1; bacon,

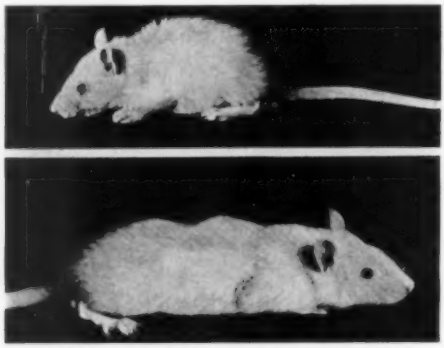

Fig. 3 Photographs of typical experimental animals. Above, negative control showing alopecia of the eye-lid and cataract. Below, animal which received 2.0 gm. fresh ham six times weekly. No cataract or alopecia was evident in this animal at any time. The rats were photographed on the fifty-ninth day on experiment, at which time they weighed 37 and 101 gm. respectively.

0.36; cured ham, 0.81; fresh ham, 1.2, and pork liver, 9.2 units per gram.

With our experimental diet, technic and strain of rats, one unit as defined above is equal to approximately 2.5 micrograms of crystalline riboflavin (Day, Darby and Langston, '37, and subsequent unpublished experiments). It therefore becomes a simple matter to translate units into the equivalent number of micrograms of riboflavin. The last column in table 1 gives the content of riboflavin in the meats studied computed in this way. To be sure, the flavin may not all have

existed in the organ or tissue as simple riboflavin; at least a part of it may have been riboflavin phosphate or a flavoprotein complex. The computed content of riboflavin must be interpreted as meaning that the meat contained a total amount of riboflavin—free and combined—biologically equivalent to the amount of riboflavin stated. Thus, 1 gm. of pork liver contained a total amount of flavin nutritionally equivalent (for the rat) to 23 micrograms of riboflavin.

DISCUSSION

Reports from several laboratories (Daniel and Munsell, '37) indicate that beef muscle (round steak) contains between 1.0 and 1.25 units of 'vitamin G' per gram of fresh meat. The lower content of riboflavin in brisket (0.74 unit per gram), as found in this investigation, is probably due to the greater amount of connective tissue and fat in this cut of meat.

Christensen, Latzke and Hopper ('36) reported that fresh pork contained more than 1.0 unit of vitamin G per gram. This agrees very well with our value of 1.2 units of flavin per gram of fresh ham (pork). The decidedly lower content of riboflavin in bacon is readily explained upon the basis of the high fat content of this pork product.

Our results indicating that lamb muscle contains 1.1 units per gram demonstrates that this meat is essentially the equivalent of beef muscle and pork muscle as a source of riboflavin in the diet. It is apparent that muscle meats from pork, beef and lamb are surprisingly similar in their content of riboflavin, each containing slightly more than 1 unit per gram.

Although data on the riboflavin content of pork liver have not previously been available, the livers of both beef and veal were found to contain approximately 10 units of vitamin G per gram (Day, '31). The content of 9.2 units per gram for pork liver, as here reported, indicates that liver from this species is essentially equivalent in flavin content. As is true in the case of muscle meats, the livers of market beef, veal and pork are surprisingly similar in their content of riboflavin.

Fresh liver, whether from beef, veal or pork, is the equivalent of many samples of dried yeast (Daniel and Munsell, '37), and on the basis of dry weight is apparently the richest known natural food source of riboflavin.

A total of forty-eight control rats was used in the series of experiments. Although the animals receiving meat supplements were killed at the end of 10 weeks, the control rats receiving only the deficient diet were retained until death. Of these, twenty-five (52%) exhibited cataract by ophthalmoscopic examination during the 10-week experimental period, but thirty-two (67%) exhibited cataract before death. The average time of appearance of cataract was 65 days, and the average survival period was 88 days.

A perusal of table 1 reveals the interesting fact that cataract appeared in the groups of animals receiving small amounts of meats, although the incidence was lower than among the negative controls. Cataract was found only rarely in the groups of animals receiving the larger amounts of meat. This is in complete agreement with our previously published statement that rats receiving a limited amount of flavin only occasionally developed cataract where the growth was 30 gm. or more in the 10-week experiment, and in no case developed cataract where the net growth was more than 56 gm. in 10 weeks (Day and Darby, '36 a). This inverse relation between riboflavin intake and incidence of cataract is exactly what would be expected in the light of our recent experiments showing that the cataract resulting from 'vitamin G' deficiency may be prevented by the feeding of pure riboflavin (Day, Darby and Langston, '37), and in its early stages can be arrested by the administration of pure riboflavin (Day, Darby and Cosgrove, '38).

SUMMARY

Using a rat-growth method, determinations of the riboflavin content of a number of meats have been made. The organs and tissues studied were found to contain the following amounts of riboflavin, expressed in units per gram of fresh

uncooked material as purchased: beef brisket, 0.74; lamb chops, 1.1; bacon, 0.36; cured ham, 0.81; fresh ham, 1.2 and pork liver, 9.2.

With our diet, technic and strain of animals, unpublished experiments indicate that 1 unit is equivalent to approximately 2.5 micrograms of crystalline riboflavin. The meats assayed were therefore found to contain amounts of 'vitamin G' biologically equivalent to crystalline riboflavin as follows: beef brisket, 1.9; lamb chops, 2.8; bacon, 0.9; cured ham, 2.0; fresh ham, 3.0 and pork liver, 23 micrograms per gram.

Of a total of forty-eight controls receiving the deficient diet only, twenty-five (52%) developed cataract during the 10-week experiment, and thirty-two (67%) exhibited cataract before death. The incidence of cataract in the groups of rats receiving meat supplements varied in inverse relation to the growth.

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FURTHER STUDIES ON DIETARY FACTORS ASSOCIATED WITH NUTRITIONAL MUSCLE DYSTROPHY

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FIVE FIGURES

(Received for publication May 9, 1938)

Morgulis and Spencer ('36) concluded that muscular dystrophy must be regarded as a multiple deficiency disease. Wheat germ being an excellent source of the essential dietary factors we fractionated this material and fed the separate fractions as an adjunct to the basal diet 13 or 313. The fractions were always administered in quantities equivalent to 20 to 30 gm. wheat germ.

FAT-SOLUBLE FRACTION

1. Ether extract of wheat germ. Both a commercial ether extracted oil and an oil which we prepared with specially purified ether was found to give greater protection than the cold pressed oil. The animals invariably become dystrophic, but the time required was occasionally as long as four months. The greater prophylactic action of this oil is attributed to the fact that continuous extraction with ethyl ether probably removes small amounts of the water-soluble fraction also.

2. Petroleum ether extract of wheat germ. On the dystrophy-producing diet supplemented with this fraction the rabbits become severely dystrophic but the time of onset is somewhat delayed, the effect being very similar to that of the cold pressed oil. The extracted residue of the wheat germ fed in conjunction with diet 13 does not prolong the

onset of dystrophy. On adding the petroleum extract to this combination, the animals have been cured and once more made dystrophic by omitting the residue.

3. Acetone extract of wheat germ. This extract has both preventive and curative properties. Rabbits receiving 2 to 3 cc. of this acetone preparation together with diet 13 were in good health and attained a body weight similar to that of control animals of the same age. The acetone extracted wheat germ, on the other hand, had no effect on the development of dystrophy showing that the acetone completely removes the essential dietary factors.

4. Hexane fraction of the acetone extract. In view of the fact that the acetone extract is complete, so far as muscle dystrophy preventing or curing factors are concerned, we fractionated this by means of hexane which removes only lipid-soluble material. This hexane fraction added to the diet 13 acted very much like the petroleum ether extract or the cold pressed oil of wheat germ. The animals invariably became dystrophic but more slowly than on diet 13 alone.

5. Non-saponifiable fraction of wheat germ oil. The non-saponifiable fraction of wheat germ oil is a source of vitamin E. Our studies with this fraction as a supplement to diet 13 show that the animals become dystrophic just as they do on cold pressed oil, the hexane fraction or the petroleum ether extract.

WATER-SOLUBLE FRACTION

The petroleum ether extracted wheat germ has no protective or curative action if fed as an adjunct to diet 13. This residue was used as the source for obtaining the water-soluble fraction by means of exhaustive extraction with 70% alcohol. The alcohol extracts were concentrated under reduced pressure and filtered, the residual aqueous solution being preserved with benzoic acid. Feeding this extract alone as a supplement to diet 13 had no effect and the rabbits quickly became dystrophic.

COMBINATION OF THE FAT-SOLUBLE AND WATER-SOLUBLE FRACTION

Neither the fat-soluble nor water-soluble fraction obtained from wheat germ can prevent or cure muscle dystrophy in rabbits fed diet 13. We studied therefore their combined effect. In figure 1 we reproduce a few representative growth curves to illustrate the effect of such combinations.

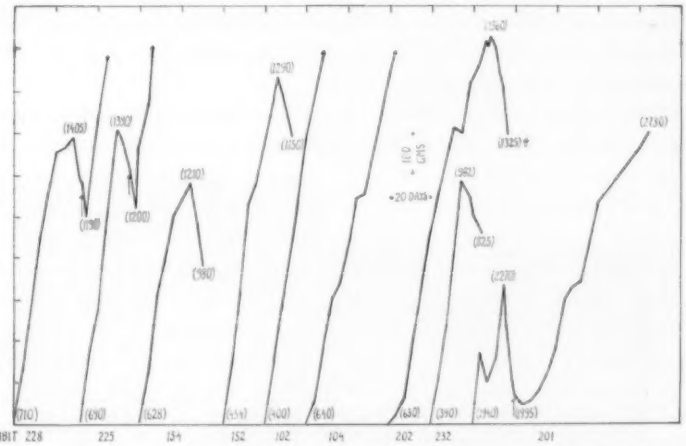

Fig. 1 Rabbit no. 154, dystrophic on diet 13. Rabbit no. 152, dystrophic on diet 313. Rabbit no. 102, normal growth on diet 313 + whole wheat germ. Rabbit no. 104, normal growth on diet 313 + acetone extract of wheat germ. Rabbit no. 202, dystrophic on diet 13 + petroleum ether extract of wheat germ (fat-soluble fraction). Rabbit no. 232, dystrophic on diet 13 + water-soluble fraction of wheat germ. Rabbit no. 201, dystrophic on diet 13 + petroleum ether extract and cured by the addition of the water-soluble fraction from wheat germ. The growth curve is plotted beginning with the twenty-third day of the experiment. Rabbit no. 228, dystrophic on diet 13 and cured by adding petroleum ether extract +5% yeast. Rabbit no. 225, dystrophic on diet 13 and cured by adding the non-saponifiable fraction of wheat germ oil and the water soluble fraction of wheat germ.

1. Petroleum ether and alcohol extracts. Animals made dystrophic on diet 13 supplemented with the petroleum ether extract from wheat germ recover from their paralysis quickly when the water-soluble fraction is added to their

ration. It has been found necessary at times to administer this extract by pipette for a few days because the sick animals were unable to eat or drink. Nevertheless, they were able to stand up after even a few administrations of the water-soluble extract.

Rabbits supplied with both petroleum ether and alcohol extracts make quick and really spectacular recovery from dystrophy, and we have kept them in good condition for a few months afterward. Most of the animals, however, have ultimately suffered a relapse. Since there was invariably a pulmonary involvement, we are not certain at this time whether the relapse which occurred 1 to 3 months subsequent to the cure was not due to an intercurrent infection.

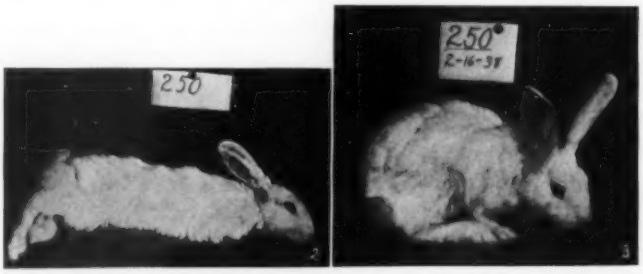

Fig. 2 Rabbit 250. Dystrophic after 24 days on diet 13.

Fig. 3 Rabbit 250 after 9 days on curative diet of diet 13 + non-saponifiable fraction of wheat germ oil + water-soluble fraction of wheat germ.

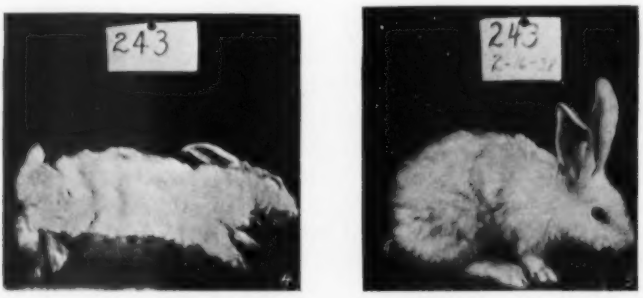

Fig. 4 Rabbit 243. Dystrophic after 22 days on diet 13.

Fig. 5 Rabbit 243 after 12 days on curative diet of diet 13 + petroleum ether extract of wheat germ + 5 gm. yeast per day.

2. Hexane fraction and alcohol extract. Three rabbits made dystrophic on the basal diet were definitely cured by adding both the hexane fraction of an acetone extract from wheat germ and the alcohol extract.

3. Non-saponifiable fraction and alcohol extract. The non-saponifiable material from wheat germ oil was fed in capsules and the water-soluble fraction of the wheat germ (alcohol extract) was given at first by pipette but later it was incorporated in the diet. Definite cures were effected by this combination as can be seen from figures 2 and 3. However, there was a recurrence after about 4 weeks. We have reason to believe that possibly mixing with the basal diet results in destruction of some of the factors, but this question will need to be studied further.

4. Petroleum ether extract of wheat germ and yeast. The water-soluble fraction has such obvious relation to the vitamin B complex that it seemed advisable to study the effect of combining our fat-soluble fraction of wheat germ with a rich vitamin B source. We chose yeast for this purpose. Figure 4 shows a rabbit which became severely dystrophic; figure 5 shows this animal 12 days later after the basal diet was supplemented with a petroleum ether extract and with 5 gm. yeast per day. Experiments now in progress with various other sources of the vitamin B complex are also proving very successful.

STUDIES ON LETTUCE

Morgulis and Spencer ('36) found that the addition of 50 gm. lettuce per day to diet 13 practically doubled the time required for the onset of dystrophy but if wheat germ oil was also added dystrophy could be prevented or cured. Since the accumulating evidence favors the view that the fat-soluble factor is closely associated, or is perhaps even identical with vitamin E, and lettuce is a good source of vitamin E, we experimented with feeding fresh lettuce ad libitum. Growing rabbits consume 300 to 500 gm. per day freely, and we found that such a supplement to diet 13 is

highly satisfactory for normal growth and maintenance of good muscle condition. We have used this supplement both as a preventive and curative measure with perfect success.

MISCELLANEOUS STUDIES

Limitations of space make it impossible to more than mention dietary studies with other foodstuffs. Studies on cottonseed meal, acetone extracts of cottonseed and the non-saponifiable fraction of the cottonseed oil show that these behave like similar preparations from whole wheat germ, but the cottonseed contains the necessary factors in smaller amounts.

Cornmeal, on the other hand, even in as high an amount as 50% of the ration, only delayed the onset of disease, the rabbits becoming severely dystrophic in about 2 months. An acetone extract as well as different commercial corn oils gave similar results. Since corn oil is a good source of vitamin E, corn either lacks or is very low in the water-soluble factor present in wheat germ, cottonseed or lettuce.

Experiments with egg yolk show that it behaves like wheat germ oil. Egg yolk as the only supplement to diet 13 cannot prevent the onset of dystrophy, but dystrophy so developed disappears rapidly upon the further addition of 50 gm. lettuce per day to the ration.

DISCUSSION AND SUMMARY

Dietary studies on the prevention or cure of nutritional muscular dystrophy in rabbits demonstrate clearly the multiple factor nature of this deficiency disease. All the essential factors are present in acetone extracts of wheat germ or cottonseed, though not in adequate amounts in the latter. Fresh green leaves of lettuce fed in large amounts with the basal diet can cure dystrophic rabbits and maintain them in good health for a long time.

Whole wheat germ was fractionated into its fat-soluble and water-soluble components. Neither fraction by itself can either prevent or cure dystrophy. The fat-soluble material,

or the non-saponifiable portion of it, incorporated into the basal diet delays the onset of dystrophy while the water-soluble fraction, either as the extract or as the residue left over from the extraction with petroleum ether, does not even affect the rapidity with which dystrophy develops on diet 13. However, when the two extracts are fed together to severely dystrophic rabbits they recover very quickly and grow vigorously. Many animals after they made good progress on these curative diets for long periods of time have had a remission of the disease. Since in every case there has been some pulmonary complication, the recurrence of dystrophy after the animals have been gaining steadily and behaved entirely normally even for months will need further investigation.

Cures have been effected not only by combining wheat germ oil and the water-soluble fraction of wheat germ but also by combining the latter fraction with a petroleum ether extract of wheat germ or with its non-saponifiable fraction, as well as by combining the fat-soluble fraction with yeast or with different vitamin B concentrates.

It may, therefore, be regarded as proved that one of the essential dietary factors is present in the non-saponifiable fraction of the wheat germ oil. The fact that this factor is absent in linseed oil but is found also in lettuce, cottonseed or corn oil, showing that its distribution is similar to that of vitamin E, and the fact that it is destroyed by the same treatment which destroys vitamin E indicate that both are either very closely associated or possibly even identical.

Pappenheimer and Goettsch ('31) and Morgulis and Spencer ('36) found that muscle dystrophy in rabbits cannot be cured by vitamin E concentrates alone but, on the other hand, foods which prevent or cure this condition contain vitamin E. We have shown that a petroleum ether extract of wheat germ or the non-saponifiable fraction of wheat germ oil as supplements to diet 13 merely delay the onset of dystrophy but do not prevent it or cure it. We tested these preparations for the vitamin E content by studying their

effect in restoring fertility in rats which have ceased to reproduce on a vitamin E-free diet, and found them to be effective sources of this vitamin. But they are effective in curing or preventing muscle dystrophy only when they are administered in combination with the water soluble fraction of wheat germ, or various concentrates of the vitamin B complex.

Judging by the distribution of the water-soluble factor (or factors?) this obviously belongs in the vitamin B complex since yeast, wheat germ and lettuce are all good sources, and experiments now in progress further substantiate this assumption. The dystrophy-producing diet itself must contain enough of the recognized growth factors since Pappenheimer and Goettsch ('31) maintained rats for three generations and we reared two generations of rats on diet 13 or 313 without any ill effects. The antineuritic vitamin B_1 is excluded automatically and B_2 (riboflavin) can also be ruled out safely because Guha ('31) has shown that this factor is not destroyed by Perhydrol (Merck) while our diet 313, which is treated with Superoxol, is as effective in producing dystrophy as is diet 13 treated with $FeCl_3$. Vitamin B_6 may likewise be disregarded because it is not extracted by acetone, while our acetone extract of wheat germ is very effective in preventing or curing nutritional dystrophy. We must, therefore, consider vitamin B_4 which Elvehjem ('35) found to be very labile, especially in the presence of oxygen. It is present in wheat, yeast and green foods (lettuce, alfalfa) just as the water-soluble dystrophy-preventive factor which we have been studying. The fact that Pappenheimer and Goettsch ('34) produced a purely myopathic condition in ducklings on the same synthetic diet which in chicks causes a myelopathic disease thought to be similar to a B_4 deficiency further suggests a possible connection between this vitamin and our water-soluble factor. However, there is one very serious objection to identifying these two dietary factors which must not be overlooked, namely, that rats do well even for several generations on the diets which produce muscle dystrophy in rabbits.

In conclusion it may be said that of the essential nutritional factors necessary for the integrity of the skeletal muscles the fat-soluble one is very closely associated or perhaps even identical with vitamin E and is present in the oil of wheat germ, cottonseed, corn, also in lettuce and alfalfa. It is also present in the non-saponifiable portion of the oil and in our ether, petroleum ether or acetone extracts of wheat germ. It can be separated from the acetone extract by means of hexane. The water-soluble fraction can be obtained by extraction of wheat germ with 70% alcohol or with acetone, and is also present in the wheat germ residue after extraction with petroleum ether. This fraction belongs to the vitamin B complex because it can be furnished by lettuce, yeast and a variety of vitamin B concentrates.

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THE EFFECT OF A HIGH GLUCOSE AND A HIGH FRUCTOSE DIET ON THE BODY WEIGHT AND ON THE FAT, GLYCOGEN, AND NITROGEN CONTENT OF THE LIVER AND BODY OF THE ALBINO RAT [1]

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ONE FIGURE

(Received for publication April 25, 1938)

The different course of the respiratory quotient following the ingestion of glucose and of fructose as reported by various workers, would indicate a marked difference in the metabolism of the two sugars. It should, therefore, be of interest to know the comparative effect of these two sugars on body weight and on the various constituents of the tissues. Feyder ('35) found that rats fed on sucrose gained weight more rapidly than litter mates on an equicaloric amount of glucose, the larger increase in weight being due to a greater deposition of fat. It would appear from these observations that the greater fattening effect obtained with sucrose should be attributed to the fructose portion of the molecule. Since, however, the results observed with sucrose might possibly represent a synergistic rather than an additive effect of the two hexoses produced by the hydrolysis of sucrose, it was deemed desirable to carry out experiments in which a direct comparison of the effects of feeding glucose and fructose could be made. Shortly after the completion of these experiments a somewhat similar study was reported by Mitchell, Hamilton and Beadles

[1] Presented before the American Physiological Society, March 30 to April 2, 1938.

('37). In our experiments, however, the liver was analyzed separately from the rest of the body; furthermore, our animals were not kept, as were theirs, in revolving cages.

METHOD

Wistar colony rats were inbred and maintained on the same diet. Litter mates—five pairs of males and five pairs of females—were selected at weaning and placed on a diet identical in every respect except that one contained glucose, the other fructose. The diet consisted of glucose or fructose 68%, commercial casein 18%, dried Fleischmann's brewers' yeast 10%, salt mixture of Osborne and Mendel 4%, and 6 drops daily of a 7:1 mixture of cod liver oil and linoleic acid. The glucose was c.p. grade and the fructose 90% pure, the remainder consisting of other sugars. The addition of linoleic acid was suggested by the observations of Burr and Burr ('29). In none of our rats was there any evidence of scaly tail or dermatitis.

The caloric value of the diets was determined with the bomb calorimeter, both before and after drying the sample to constant weight. Before drying, the heat of combustion of the glucose and fructose diets was respectively 4.034 and 4.068 calories per gram; after drying, 3.967 and 3.985 calories.

Upon weaning, the animals were put in separate cages and placed immediately on the experimental diet. Their activity was limited by the size of the cages which measured $17.5 \times 20 \times 27.5$ cm. The food was kept in conical shaped cups (Franke's) to minimize spilling and, as far as could be determined, the total amount spilt in feeding was inappreciable. Each animal was weighed daily. The paired feeding method (Mitchell and Beadles, '30) was followed, the food intake of the two animals being completely equalized once a week and at the conclusion of the experiment. Since fructose is deliquescent, each animal's diet was kept in a separate bottle tightly corked. On 'balance days' the total amount of food consumed up to this time was determined by weighing the bottles.

The animals were kept on the experimental diet for 70 days. They were then fasted for 24 hours, weighed and killed by a blow on the head. The liver was removed immediately, the blood squeezed out and the organ cut into several pieces and dropped into a tared vessel containing 30% cold potassium hydroxide (compare Cori, '32). The remainder of the animal was quickly chopped with a cleaver and immersed in boiling caustic. The tissues were in the caustic in less than 1 minute after death. Both the liver and the body were analyzed for fat, nitrogen, and glycogen. The fat content was determined

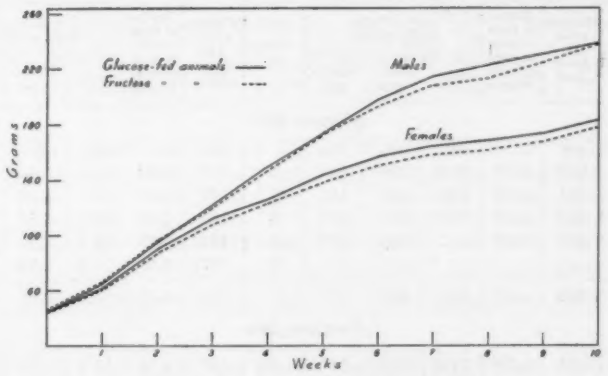

Fig. 1 Body weight on high sugar diets.

by the method of Leathes and Raper ('25) as described by Feyder ('35); nitrogen by the macro-Kjeldahl method and glycogen by the method of Good, Kramer and Somogyi ('33).

RESULTS

Body weight. The average weekly increase in weight is shown in figure 1. The averages for the males were derived from only four animals since one on the glucose diet died (from an unknown cause) toward the end of the experiment. The average weekly increase in weight of the paired animals, as shown in the figure, was uniformly the same on the glucose and fructose diets. At the conclusion of the experiment, the

total increase over the original weight at the time of weaning for the glucose and fructose-fed males was 431 and 429%, respectively; for the females 289 and 287%. The females required appreciably more food than the males for an equal gain in body weight, the mean ratio on the glucose and fructose diets being 5.35 and 5.51, respectively, as compared with 4.42 and 4.44, respectively, for the males.

TABLE 1

Analytical data on body of rats on glucose and fructose diets

MALES						FEMALES					
Rat no.	Weight after 24-hour fast	Ratio of food intake to gain in body weight	Per 100 gm. body weight			Rat no.	Weight after 24-hour fast	Ratio of food intake to gain in body weight	Per 100 gm. body weight		
			Fat	Nitrogen	Glycogen				Fat	Nitrogen	Glycogen
Glucose diet											
	gm.		*gm.*	*gm.*	*mg.*		*gm.*		*gm.*	*gm.*	*mg.*
1	238	3.74	20.3	2.88	147	6	167	4.10	16.0	3.43	169
2	247	4.33	18.2	2.89	147	7	170	6.13	17.5	2.94	168
3	223	4.53	13.8	3.05	162	8	180	5.29	20.5	2.74	162
4	205	5.08	...	3.28	224	9	164	5.68	16.1	2.89	185
						10	177	5.57	14.1	2.98	165
Av.	228	4.42	17.4	3.02	170		172	5.35	16.8	2.99	169
Fructose diet											
1a	238	3.78	14.4	2.98	121	6a	162	4.19	17.4	3.24	138
2a	251	4.14	16.4	2.80	151	7a	175	6.56	15.6	2.76	140
3a	206	4.99	11.0	3.83	187	8a	169	5.75	13.8	2.87	172
4a	214	4.84	...	2.85	226	9a	177	5.24	10.8	2.97	231
						10a	169	5.83	12.3	2.92	211
Av.	227	4.44	13.9	3.11	171		170	5.51	14.0	2.95	178

Analysis of the body. The average total glycogen and nitrogen content of the entire body, as shown in table 1, was the same per 100 gm. of body weight in the glucose as in the fructose-fed animals. The amount of fat, however, was greater in the glucose-fed animals in every instance except one. The total body fat given in the tables as percentage of body weight was, respectively, 8.8 and 5.2 gm. more in the males and females fed on glucose than in their litter mates on

fructose, or an average difference of 6.5 gm. for the entire group. The average percentage of body fat of the males and females combined was 17.1 on the glucose diet as compared with 14.0 for the animals fed fructose. The difference of 3.1 between the percentages was statistically significant, as P (calculated by Fisher's method, '28) was 0.007. The greater amount of fat in the glucose-fed animals was therefore due

TABLE 2

Comparison of the liver of rats on glucose and fructose diets

MALES						FEMALES					
Animal no.	Weight of liver	Weight per 100 gm. body weight	Per 100 gm. liver			Animal no.	Weight of liver	Weight per 100 gm. body weight	Per 100 gm. liver		
			Fat	Nitrogen	Glycogen				Fat	Nitrogen	Glycogen
Glucose diet											
	gm.	*gm.*	*gm.*	*gm.*	*mg.*		*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
1	5.832	2.45	5.25	3.57	309	6	4.056	2.43	5.92	3.70	232
2	6.642	2.70	5.06	3.64	754	7	5.383	3.17	5.39	3.53	372
3	5.474	2.45	5.11	4.20	873	8	4.913	2.73	5.29	3.56	381
4	5.867	2.86	4.26	3.56	954	9	5.067	3.09	5.53	3.47	227
						10	5.617	3.17	4.98	3.49	454
Av.	5.954	2.62	4.92	3.74	721		5.007	2.92	5.42	3.55	333
Fructose diet											
1a	7.291	3.07	4.39	3.43	191	6a	4.978	3.07	5.02	3.62	100
2a	8.216	3.27	4.14	3.61	353	7a	6.215	3.55	4.18	3.65	183
3a	6.947	3.37	3.74	3.63	999	8a	6.167	3.65	4.05	3.57	316
4a	6.633	3.10	4.31	3.68	638	9a	6.998	3.95	3.57	3.02	1479
						10a	5.890	3.49	3.94	3.57	955
Av.	7.272	3.20	4.15	3.59	545		6.050	3.54	4.15	3.50	607

to a specific effect of the glucose. These results, while seemingly contradictory to those reported by Mitchell, Hamilton and Beadles are not strictly comparable with theirs, inasmuch as their glucose and fructose-fed animals, unlike those in the present study, were kept in revolving cages.

Weight of the liver. The weight of the liver is shown in table 2. In every instance it was heavier in the fructose-fed animals, being 22 and 21% larger in the males and females,

respectively. As this could have happened as a chance occurrence only twice in 512 experiments (Fisher, '28, p. 114) the larger liver of the fructose-fed animals, as compared with that of the glucose-fed animals, must be attributed to the fructose. The average weight of the liver of the glucose and fructose-fed males expressed as percentage of body weight was 2.62 and 3.20, respectively, and that of the females 2.92 and 3.54, respectively.

Analysis of the liver. The percentage nitrogen of the liver showed no significant difference with the two diets (table 2). The glycogen content was extremely variable among the different animals. The average was higher than that reported by Cori and Cori ('28) and by Wilson and Lewis ('29–'30). After a 24-hour fast, the first-named authors obtained an average of 0.2% and in a later series of experiments ('29) an average of 0.1%; Wilson and Lewis an average of 0.21%. As we obtained similarly low values in two animals taken at random from our colony and fed on a stock diet before fasting, it would appear that the high glycogen content of the liver in the present experiments was due to the large amount of carbohydrate in the diet. In this connection it should be noted that Deuel and co-workers ('34) found an average of 1.43 and 0.70% in the liver of males and females, respectively, on a high carbohydrate diet. Our grand averages obtained by grouping the males and females showed 74 mg. more glycogen per 100 gm. of liver tissue on the fructose than on the glucose ration, but the difference was of no statistical significance ($P > 0.3$).

The percentage of fat in the liver was higher in the glucose-fed animals in every experiment except one, and in this instance it was practically the same in the paired animals on the two diets (rats nos. 4 and 4a). The difference in the average percentage of fat in the liver of the glucose and fructose-fed rats was highly significant with $P = 0.00025$. Notwithstanding this difference in percentage, the absolute amount of fat in the liver was practically the same on the two diets (280 and 272 mg.). This can be explained by the circumstance that the liver of the fructose-fed rats was heavier.

DISCUSSION

It is somewhat difficult to account for the fact that the average weight of the animals was the same on the two rations despite a larger amount of fat in the glucose-fed animals. The water content of the tissues could not be directly determined owing to the procedure required for the analysis of glycogen, but when the total amount of fat, protein ($N \times 6.25$) and glycogen was subtracted from the body weight, the remainder was larger by 4 gm. in the fructose than in the glucose-fed animals. Since this difference is too great to be accounted for by any conceivable difference in the sugar content of the animals and inorganic salts, it would seem to indicate a greater degree of hydration of the tissues of the fructose-fed animals.

It would be of interest to know why the animals on the fructose ration had a larger liver than those on glucose. Since the liver of the fructose-fed rats had practically the same percentage protein content as that of the glucose-fed rats, namely, 22.6 as compared with 22.1%, we are forced to conclude that its greater weight was due to an increase in the parenchyma of the organ. This enlargement of the liver may have been the consequence of an increased functional demand on the organ in relation to the metabolism of fructose. More work is probably done by the liver in the formation of glycogen from fructose than from glucose because of the more indirect route by which the former is converted into glycogen. Furthermore, it has been shown that in man blood lactic acid rises shortly after the ingestion of fructose, whereas this does not occur within 30 minutes after taking the same amount of glucose (Campbell and Maltby, '28; Bachmann and Haldi, '37). The liver removes lactic acid from the blood (Himwich, Koskoff and Nahum, '28, '30; McClure, '31, '32) and the lactic acid so removed is converted into glycogen (Cori and Cori, '29). If lactic acid is formed in the rat after the ingestion of fructose, its removal would probably entail additional work on the part of the liver.

SUMMARY

Litter mates of albino rats were fed for 10 weeks on equicaloric diets of the same composition except that one contained 68% glucose, the other the same percentage of fructose. The activity of the animals was limited by the restricted size of the cages in which they were kept.

The average increase in body weight on the two diets was the same. The ratio of food intake to gain in weight was therefore the same for the two sugars; it was, however, considerably higher for the females than for the males.

The average total glycogen and nitrogen percentage content of the entire body was the same in the glucose and fructose-fed animals. The fat content was significantly greater in the rats on the glucose diet. The hydration of the tissues was apparently greater in the fructose-fed animals.

The liver of the animals on the fructose diet was heavier in every experiment, the average weight for the group being 22% greater than in the glucose-fed animals. This hypertrophy of the liver was probably due to an increase in the work of the organ in the metabolism of fructose.

There was no significant difference in the percentage nitrogen and glycogen content of the liver with the two diets. The percentage fat content was significantly greater in the liver of the glucose-fed animals. The absolute amount of fat in the organ, however, was the same with the two diets. This is accounted for by the fact that the liver of the fructose-fed animals was heavier.

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COMPARATIVE EFFECTS OF A HIGH GLUCOSE AND A HIGH FRUCTOSE DIET ON ACTIVITY, BODY WEIGHT AND VARIOUS CONSTITUENTS OF THE LIVER AND BODY OF THE ALBINO RAT EXERCISING AT WILL[1]

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TWO FIGURES

(Received for publication April 25, 1938)

The purpose of the present experiments was to study the activity and growth of the albino rat when fed on glucose and on fructose and allowed to exercise at will in revolving cages, together with the same constituents of the body and liver as in the preceding experiments. The procedure was the same except that the animals, when weaned, were placed in separate small cages and 10 days later removed to individual revolving cages for the remainder of the experiment. These cages were equipped with two Veeder ratchet counters, one attached to each end of the shaft in order that rotation in either direction might be recorded. The resistance of the wheel was negligible, the shaft rotating on ball bearings. Forty-one animals were used: sixteen on the glucose ration, sixteen on fructose and nine on the mixture of the sugars.

RESULTS

Body weight. The average weight of the males on the different rations is shown at weekly intervals in figure 1. Each

[1] Presented before the American Physiological Society, March 30 to April 2, 1938.

curve represents an average of six experiments. The gain in weight on the glucose and fructose rations from week to week was practically the same, except at the end of the fourth and fifth weeks when the glucose-fed animals were 6 and 8 gm. heavier. This was probably an accidental variation. At the end of the experiment the glucose-fed animals (ten experiments) showed an average increase of 505% and the fructose-fed animals 513% over the original weight.

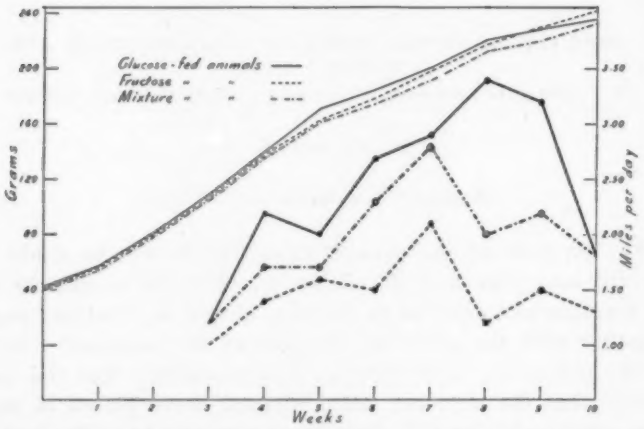

Fig. 1 Body weight and spontaneous activity of male rats on high sugar diets. (The curves of activity are shown in bold lines and the points on the curves mark the average activity of the preceding week.)

In figure 2 is shown the average weekly gain in weight of female litter mates on glucose and on fructose. Each average was obtained from six experiments. The weight of the females on the mixture was not plotted as there were only three animals in the series. The increase in weight was consistently greater on glucose than on fructose. The percentage increase, however, was not as large as the curves would seem to indicate as the glucose-fed rats were 1 gm. heavier at the beginning of the experiment. At the conclusion of the experiment the respective gains for the animals on glucose and fructose

were 388% and 383%. When the males and females were combined (sixteen experiments), the glucose-fed animals showed an average gain of 180 gm. as compared with 176 gm. for those on fructose. The difference was statistically of no significance ($P = 0.27$).

The average ratio of food intake to gain in body weight for ten males was 4.37 and 4.41 on the glucose and fructose rations, respectively, and for six females 5.35 and 5.54, respectively.

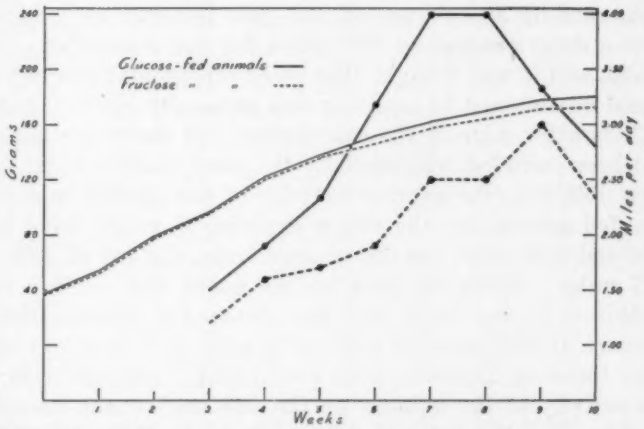

Fig. 2 Body weight and spontaneous activity of female rats on sugar diets.

Activity. The average number of miles run per day is shown for each week in figures 1 and 2, each point on the curves representing the miles run in the preceding week. The distance was obtained by multiplying the circumference of the wheel by the total number of revolutions. The average activity of both the males and females on the glucose ration was greater for each of the 8 weeks than that of their litter mates on fructose. The activity of the animals on the mixture was intermediate. The glucose, fructose and mixture-fed males ran an average of 2.42, 1.44 and 1.95 miles daily, or a total during the experiment of 145, 86 and 117 miles. The

glucose and fructose-fed females ran an average of 2.87 and 2.07 miles daily, or a total of 172 and 124 miles, respectively. The glucose-fed animals were more active 64 weeks, the fructose-fed animals 24 weeks, if the total activity of the eleven pairs of animals active for 8 weeks be regarded as the equivalent of 88 weeks.

The analytical data on the females in table 1 were derived from six animals, whereas the activity curves in figure 2 were plotted from the averages of only five animals. The reason for this discrepancy is that one animal was extremely sluggish, running an average of 0.65 mile per day as compared with a daily average of 2.66 miles for the remainder of the group, and it was thought that more representative averages would be obtained by omitting this unusually inactive animal and its litter mate in the calculations. If these two animals had been included, substantially the same results would have been obtained; the average activity of the glucose and fructose-fed groups for the entire experiment would have been 2.50 and 2.16 miles per day, respectively, instead of 2.87 and 2.07 miles. When the data on the males and females were combined, it was found that the glucose-fed animals ran an average of 0.89 mile per day (or a total of 53.4 miles) more than those on fructose, with $P = 0.0024$. The difference in the activity of the animals on the two sugars was therefore significant. Mitchell, Hamilton and Beadles also found greater activity manifested by their glucose-fed animals. Their experiments, however, were not as conclusive, as P in their calculations was equal to 0.044.

Analytical data. Table 1 contains the analytical data on all the animals. There are two sets of averages, one for the nine animals on each of the three diets and one for the sixteen on glucose and the same number on fructose. It is worthy of note that the averages of the various tissue constituents were substantially the same whether they were obtained from nine or sixteen experiments.

Analysis of the body. The nitrogen content of the body was practically the same in the glucose, fructose and mixture-fed rats. The same was true of the glycogen content. The

TABLE 1

The body and liver of rats on high glucose and high fructose diets

ANIMAL NO.[1]	DIET	BODY				LIVER				
		Weight after 24-hour fast	Per 100 gm. body weight			Weight	Weight per 100 gm. body weight	Per 100 gm. body weight		
			Fat	Nitrogen	Glycogen			Fat	Nitrogen	Glycogen
		gm.	gm.	gm.	mg.	gm.	gm.	gm.	gm.	mg.
1 ♂	Glucose	213	6.39	3.30	142	6.585	3.10	4.77	3.61	118
2 ♂	Fructose	242	8.95	3.20	90	8.742	3.61	4.21	3.76	81
3 ♂	Mixture	232	8.06	3.23	136	7.612	3.28	3.81	3.60	1097
4 ♂	Glucose	230	12.67	3.01	74	6.426	2.80	4.92	3.61	132
5 ♂	Fructose	243	9.74	3.06	75	7.844	3.23	4.00	3.67	249
6 ♂	Mixture	214	12.54	2.98	110	6.315	2.95	4.47	3.80	728
7 ♂	Glucose	250	11.62	3.20	77	7.271	2.91	4.18	3.55	138
8 ♂	Fructose	196	13.12	2.95	138	6.798	3.47	3.94	3.53	883
9 ♂	Mixture	215	16.13	2.93	136	6.906	3.21	4.00	3.71	905
10 ♂	Glucose	243	8.75	3.19	110	6.761	2.79	4.44	3.61	389
11 ♂	Fructose	244	9.98	3.06	99	7.737	3.17	3.90	3.61	489
12 ♂	Mixture	233	6.22	3.14	101	6.841	2.93	3.80	3.65	436
13 ♂	Glucose	202	12.13	3.07	141	5.791	2.87	4.35	3.56	1140
14 ♂	Fructose	234	10.59	2.93	142	7.205	3.08	4.22	3.54	364
15 ♂	Mixture	225	8.17	3.11	136	6.625	2.94	3.71	3.50	1208
16 ♂	Glucose	204	8.96	3.18	125	5.943	2.91	4.01	3.63	441
17 ♂	Fructose	198	9.14	3.02	124	6.381	3.22	3.95	3.63	384
18 ♂	Mixture	204	9.84	3.10	108	6.052	2.97	4.10	3.56	400
19 ♀	Glucose	192	4.67	3.08	71	5.454	2.84	5.91	3.52	193
20 ♀	Fructose	163	4.94	3.18	92	6.368	3.91	3.99	3.61	369
21 ♀	Mixture	180	12.00	2.97	120	6.176	3.43	4.31	3.53	1117
22 ♀	Glucose	142	5.63	3.20	96	4.853	3.42	4.45	3.46	466
23 ♀	Fructose	170	14.40	2.86	98	5.247	3.09	4.54	3.58	74
24 ♀	Mixture	163	11.30	2.95	123	5.461	3.35	4.17	3.53	251
25 ♀	Glucose	152	12.53	2.91	120	4.829	3.18	5.54	3.32	32
26 ♀	Fructose	150	5.70	3.12	104	5.827	3.89	3.95	3.48	779
27 ♀	Mixture	139	6.96	3.04	132	4.735	3.40	4.22	3.49	646
28 ♂	Glucose	229	10.40	3.06	125	7.167	3.13	4.29	3.44	781
29 ♂	Fructose	228	11.59	3.05	144	7.259	3.19	3.97	3.55	375
30 ♂	Glucose	241	16.16	2.98	96	6.679	2.77	4.19	3.52	287
31 ♂	Fructose	205	9.97	2.96	104	7.107	3.47	3.94	3.46	348
32 ♂	Glucose	218	8.80	3.11	171	5.899	2.71	3.86	3.31	280
33 ♂	Fructose	202	5.20	3.18	194	6.292	3.11	3.44	3.54	795
34 ♂	Glucose	263	9.90	3.14	162	6.873	2.61	4.39	3.46	196
35 ♂	Fructose	240	10.78	3.14	171	7.778	3.24	3.91	3.52	476
36 ♀	Glucose	185	13.87	2.87	107	5.487	2.97	5.94	3.58	97
37 ♀	Fructose	172	9.13	2.91	84	6.312	3.67	4.53	3.46	142
38 ♀	Glucose	169	11.15	3.04	117	5.236	3.10	6.30	3.44	
39 ♀	Fructose	142	7.16	3.01	165	5.017	3.53	3.47	3.47	
40 ♀	Glucose	193	11.20	2.95	68	5.928	3.07	5.57	3.37	287
41 ♀	Fructose	176	8.22	3.04	64	5.850	3.32	4.72	3.52	89
Av. 9 animals	Glucose	203	9.26	3.13	106	5.990	2.98	4.73	3.54	339
	Fructose	204	9.62	3.04	107	6.905	3.41	4.08	3.60	408
	Mixture	201	10.14	3.05	122	6.303	3.16	4.07	3.60	754
Av. 16 animals	Glucose	208	10.30	3.08	113	6.074	2.95	4.82	3.50	332[2]
	Fructose	200	9.29	3.04	118	6.735	3.39	4.04	3.56	393[2]

[1] Arranged in groups of litter mates.

[2] Average of fifteen animals.

largest difference in the body glycogen was between the animals on glucose and the mixture ration, but this difference (16 mg. %) was of no statistical significance ($P = 0.22$).

The average percentage of fat in the body of the first nine rats in the table was slightly higher in the fructose than in the glucose-fed animals. This relationship was reversed in the average of sixteen animals, indicating that the difference was an accidental variation. On the mixture ration the percentage was higher than on glucose or on fructose. Statistically, the difference between the glucose and fructose-fed groups on the one hand ($P = 0.16$), and the glucose and mixture-fed groups on the other hand ($P = 0.29$), was not significant. These results are in contrast with those of the non-exercise experiments in which there was an appreciably higher body fat content in the glucose-fed rats. In the experiments of Mitchell, Hamilton and Beadles ('37) the body fat in the glucose and fructose-fed rats was 13.8 and 11.8 gm., respectively, per 100 gm. body weight, the difference being statistically significant. These results and ours are not necessarily contradictory, since our animals were in the revolving cages for a longer time than theirs.

Liver. The liver of the fructose-fed animals was heavier than that of their litter mates on glucose in thirteen out of sixteen experiments. The liver of the mixture-fed animals was heavier than that of the glucose-fed in six out of nine experiments and lighter than that of the fructose-fed rats in seven out of nine experiments, the average weight being intermediate between that of the glucose and fructose-fed groups. The liver of the glucose-fed animals (sixteen experiments) was 2.95% of the body weight and that of the fructose-fed animals 3.39%. The difference was significant ($P = 0.0047$). Fructose, therefore, in the exercising as in the inactive rats, had the effect of producing a larger liver than glucose.

The percentage nitrogen in the liver was the same with the three diets. The glycogen content, as in the previous experiments, was extremely variable even among animals on the

same diet. The percentage glycogen was higher on the fructose than on the glucose ration but the difference was statistically of no significance ($P > 0.3$). On the mixture ration in seven out of nine experiments the percentage of liver glycogen was larger than on the glucose or fructose rations. This higher percentage in each instance was statistically significant ($P = 0.012$ and 0.022, respectively). The higher glycogen content of the liver on the mixture ration may perhaps be accounted for by differences in the rate of absorption of the various rations during the terminal fast. With a slower rate of absorption of the mixture of the sugars, as suggested by the observations of Cori ('26), glycogen would continue to be formed during the fast for a longer time than with glucose or fructose alone.

The percentage of liver fat was 4.82 in the glucose-fed rats (average of sixteen experiments) as compared with 4.04 in the fructose-fed animals. This difference was significant ($P = 0.0001$). Notwithstanding the relatively large difference in percentage fat content, the total fat in the liver on the glucose ration, owing to the smaller size of the organ, was only 20 mg. more than on the fructose ration. These observations on liver fat were practically the same as in the non-exercise experiments of the preceding paper.

DISCUSSION

A comparison of the growth curves of these and the preceding experiments shows practically no difference at the conclusion of the experiments in the body weight of the exercising and non-exercising animals. Since the original weight of the former was lower at the beginning of the experiment, their percentage gain in weight was considerably larger. The data on the food consumption show that the exercising animals ate more than the non-exercising animals. It would therefore appear as if exercise stimulated the appetite to such an extent that the animals ate not only enough additional food to supply energy for the exercise but also enough to gain more weight than the non-exercising animals. The ratio of food

intake to gain in weight in these exercise experiments was 4.37 and 4.41, respectively, for the males on the glucose and fructose rations, and 5.35 and 5.54, respectively, for the females. These ratios are practically identical with those in the non-exercising experiments. This would seem to indicate that equal amounts of food were required for the same gain in body weight when the animals ran 1½ to 2¾ miles per day as when they took practically no exercise. When, however, body fat, glycogen and protein ($N \times 6.25$) were added it was found that there was an average of 10 gm. more organic material in the body of the non-exercising animals. It would appear, therefore, as if the larger percentage gain in weight of the exercising animals was probably due to a greater hydration of their tissues. Unfortunately it was impossible to obtain direct data on this question.

Since there was more fat but approximately the same amount of glycogen and protein in the non-exercising than in the exercising animals, it would appear that the energy for the activity of the latter was at the expense of body fat in the sense that the energy required for exercise would have been stored as fat had the exercise not been taken. The average amount of fat in the non-exercising in excess of that in the exercising animals, namely 13 gm., would in all probability have sufficed for the energy required for the exercise of the most active animals, that is, those on the glucose ration. This amount of fat could have furnished 123.5 calories. If the rats had a muscular efficiency of 30%, there would have been available for the work of running, 37 calories or the equivalent of 15,784 kilogram-meters of work. If the animals had lifted their bodies to a height equal to the entire distance run, the total amount of work done, obtained by multiplying the mean weight by the average distance run from week to week would have been 31,608 kilogram-meters. Inasmuch as the animals ran in an almost horizontal position, it is reasonable to suppose that more than sufficient energy for running would have been supplied by 13 gm. of fat. Similar calculations show that the greater activity of the glucose-fed animals can account for the slight difference in the fat content

of the glucose and fructose-fed animals in these experiments, in contrast to the larger amount of fat in the glucose than in the fructose animals in the non-exercising experiments of the preceding paper. The glycogen content of the body of the exercising animals was approximately 60 mg. % less than in the non-exercising animals, but this difference could account for only a small part of the energy required for exercise.

From the data of these experiments no explanation can be offered for the greater activity of the animals on the glucose ration. It would be of interest to know whether it is related to possible quantitative or qualitative differences in the metabolism of the central nervous system on the glucose and fructose rations.

SUMMARY

Spontaneous activity, gain in weight and various constituents of the body and liver have been studied on rats maintained on diets that were identical except that one contained 68% glucose, one the same percentage of fructose and another a mixture of the two sugars in equal parts. The animals were kept in cages allowing free access to a wheel provided with counters.

There were no significant differences in the gain in weight on the three diets.

Spontaneous activity was greatest on the glucose ration, least on fructose and intermediate on the mixture.

There was but a small difference in the body fat of the glucose and fructose-fed rats of these experiments whereas in the non-exercising experiments reported in the previous paper, there was considerably more body fat on the glucose ration. The fat content of the body of both the glucose and fructose-fed animals was considerably less than in the corresponding non-exercise experiments. These differences are accounted for by the activity of the exercising animals. It is concluded that the energy required for the exercise was supplied for the most part at the expense of body fat.

The percentage nitrogen and glycogen of the body was the same on the glucose and fructose rations.

The liver was appreciably larger on the fructose than on the glucose diet.

The percentage nitrogen of the liver was the same on the two diets, and the percentage glycogen the same on glucose and fructose but higher on a mixture of the sugars; the percentage fat was higher on the glucose than on the fructose diet.

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THE EGG-REPLACEMENT VALUE OF THE PROTEINS OF CEREAL BREAKFAST FOODS, WITH A CONSIDERATION OF HEAT INJURY

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(Received for publication March 3, 1938)

Over 500,000 tons of cereal breakfast foods are being consumed by the people of the United States every year (Bureau of the Census, '35). It is time more attention was paid to the effect of manufacturing processes on the natural nutrient qualities of these foods. Morgan and King ('26) were the first to show that heating cereal proteins reduces their nutritive value as measured by the rat growth method. Their series included three of the breakfast cereals used in this study, one of which exhibited the heat injury. Morgan ('31) extended these observations on rats to raw and toasted wheat gluten; also to raw, water-cooked and toasted whole wheat, raw and puffed rice, raw yellow corn and toasted corn flakes. In each case toasting lowered the biological value very materially while differences in digestibility were very slight.

Having been interested in this class of foods for some years, this laboratory sought and obtained financial support[1] for a study of a number of the most commonly used wheat breakfast foods and the most commonly used oat product. The primary object was to determine in a more extensive way the egg-replacement value of cereal proteins. We were also interested to see whether any of the commonly used cereals would exhibit the heat injury when fed to human subjects. It

[1] We are indebted to the Cream of Wheat Corporation for material aid in the support of this investigation.

was important that the method used should approximate the conditions under which the cereal breakfast foods are consumed in American families. The use of the replacement-value method (Murlin and Mattill, '38; Sumner, Pierce and Murlin, '38) permitted this. There is growing evidence (Mitchell and Hamilton, '29; Sumner and Murlin, '38) that the biological values of foods are not the same for different species, even when the methods applied are practically identical. It seemed possible, therefore, that a reduced value for the rat might not be apparent for man. Whole egg protein uniformly has shown the highest biological value in rats (Sumner, '38) and in human subjects (Sumner and Murlin, '38). It is also much more acceptable for a group of young men subjects than is milk. For these reasons it was chosen as the reference or standard protein.

The cereals selected, using descriptive names (not trade names) and the methods of preparation so far as these could be obtained from the manufacturers, were the following:

1. Torn wheat [2] is first steam cooked under pressure, transferred to steel tanks to cool and 'mellow,' then squeezed through rollers under 1700 pounds pressure per square inch, made into 'biscuits' and finally baked to brown color.
2. Inflated wheat [3] is steam cooked under pressure in a closed autoclave-like vessel called a 'gun.' Heating occupies about 7 minutes and the interior gun temperature reaches 390°F. At this point the pressure is suddenly released causing expansion of the moisture within the berry. It is then dried to 2% moisture and packaged.
3. Toasted 'whole' wheat.[4] All the manufacturer was willing to say is that this product contains nothing but wheat. He would not say that it contains all the wheat. It obviously has been granulated, is lightly toasted and has had some bran (at least) removed.
4. Flaked wheat.[5] The grain is 'scoured' to remove beard, fine dust and loose particles of the outer bran, then is steam

[2] 'Shredded Wheat' manufactured by National Biscuit Company.
[3] 'Puffed Wheat' manufactured by Quaker Oats Company.
[4] 'Wheatena' manufactured by Wheatena Company.
[5] 'Wheaties' manufactured by General Mills Company.

cooked at 220°F. after addition of cane sugar (7%) salt (3%) and malt syrup (0.5%). It is then dried and flaked by passage between warmed rollers and finally toasted in gas-fired ovens, "where the flakes are heated for less than 1 minute to a temperature not exceeding 450°F."

5. Granulated 'whole' wheat,[6] contains all of the berry except the coarser parts of the bran. It is finely granulated to insure quick cooking, and has 5% extra wheat germ added. The final mixture is heated to kill weevil eggs before packaging.

6. Rolled oats [7] is a whole grain prepared by steaming sufficiently to cook partially, then rolled cold. There is no toasting.

7. Wheat endosperm [8] is an evenly granulated product, containing the smallest traces possible of the germ and bran. It is the coarser stage of so-called 'patent' white flour, heated only enough to sterilize.

It is evident from these descriptions and the analyses given in table 1 that two of the products, torn wheat and inflated are very nearly truly whole wheat. The protein content of the latter indicates a hard, winter, high-gluten wheat, and its high fiber indicates that it contains all the bran. On the other hand, the low protein of torn wheat indicates a soft wheat, but fiber indicates all the bran. Toasted 'whole' wheat seems to be a soft low-gluten wheat with some bran removed. Flaked wheat is made from "a whole wheat of a definite type and variety," but quite evidently not a very high-protein variety. A portion of the bran is removed. Granulated 'whole' wheat is made from a 'hard, high-protein winter wheat.' We have assumed a protein content of 16% in the whole grain and a protein content of 36% in the wheat germ added. All determinations for nitrogen in the foods consumed were made at least in triplicate and often in quadruplicate, and by the same method as that used for nitrogen in the urine and feces.

Every attempt was made to secure uniformity in the foods served. For example, the cream came from a single high-class dairy herd. Butter was churned in several separate

[6] 'Ralston's Wheat Cereal' manufactured by Ralston Purina Company.
[7] 'Mothers Quick Oats' manufactured by Quaker Oats Company.
[8] 'Cream of Wheat' manufactured by Cream of Wheat Corporation.

lots from cream produced by the same herd. The eggs which came from one flock of white Leghorn hens, fed uniformly throughout the study, were delivered every second day. Canned applesauce [9] was found very uniform in composition and was used exclusively as the only fruit for the first nine periods. Thereafter it was used to supplement fresh fruit (strawberries, raspberries and banana) in order to equalize

TABLE 1

Analyses[1] *of seven cereals used in replacement value studies*

Constituent	*Wheat endosperm*	*Torn wheat*	*Inflated wheat*	*Flaked wheat*	*Toasted 'whole' wheat*	*Granulated 'whole' wheat*	*Pre-cooked oats*
Moisture	9.35	5.62	4.90	2.72	7.06	8.08	7.36
Ash	0.50	1.79	1.40	4.97	1.35	1.45	1.84
Protein							
N × 6.25	11.56	10.93	15.06	11.69	11.25	16.32	16.13
N × 5.7	(10.54)	(9.97)	(13.74)	(10.65)	(10.26)	(14.87)	
Carbohydrate	76.96	78.15	74.26	77.10	76.35	70.61[4]	67.63[4]
Ether extract	0.85	1.35	1.94	1.49	1.89	1.47	6.14
Crude fiber	0.27	2.42	2.34	1.88	2.10[3]	2.07[3]	0.90[3]
Total	99.49	100.26	99.90	99.89	99.05	100.00	100.00

[1] Each set of figures represents at least three samples from as many packages and each done in triplicate. We are indebted to Dr. H. B. Pierce, formerly of this laboratory, for the analyses of the first four cereals; the analyses of the other three are taken from paper I (Murlin, Line, Piper and Pierce, '29) supplemented by certain more recent data obtained in connection with the present study and from the literature.

[2] Estimated.

[3] From Sherman.

[4] CH by difference.

carbohydrate in cereal and egg periods. The change to fresh fruit was necessary to preserve a good morale in the diet squad. For the first nine periods also orange juice was squeezed fresh every morning from fruit supplied [10] by special arrangement through Dr. Estelle E. Hawley of the department of pediatrics. Grapefruit juice and the mixed [11] grapefruit and orange juice were used at various times. Cornstarch

[9] 'Musselman's.'

[10] By the California Fruit Growers' Association.

[11] 'Dr. Phillips' brand.

was purchased locally in bulk by the Strong Memorial (University) Hospital. Iceburg lettuce came from the Imperial Valley, California, so long as that supply lasted after which the same variety was purchased locally. The N content was the same in the two geographical varieties. Coffee [12] was uniformly ground and brewed. Beer, which was permitted to two members of the diet squad in place of coffee, was a local product.[13]

THE DIET SQUAD

The squad for this study was composed entirely of young men identified with the medical school either as medical or graduate students or instructors. All had had some experience in research work and therefore were in a position to appreciate the necessity of strict adherence to the diet and of absolutely quantitative collection of urine and feces. For this reason we have perfect confidence in the reliability of the data.

All members of the diet squad were in perfect health and continued so throughout the period of 10 weeks on a monotonous, if perfectly adequate, dietary regime. The heights, weights and surface areas of the individual members of the squad are given in table 2, together with the average total and distributed calories for the entire period of 10 weeks. Weights fluctuated, as they naturally do, but losses never were excessive. Those who lost most weight desired to do so. Notwithstanding that a 5-day adjustment period preceded the first egg period, some alteration of total calories and very minor alterations of nitrogen intake were made from time to time in order to make the diet perfectly acceptable to the members of the squad. One member, V.C., of the squad was obliged for an emergency reason to drop out at the end of the fourth egg period. Fortunately we had one reserve member, M.S., who came into the squad with the second cereal period, and he replaces V.C. in the data reported from the fourth cereal.

[12] Chase and Sanborn brand.

[13] Genesee brand.

SUITABILITY OF THE DIET

This study was designed to establish the value of each cereal breakfast food as a source of protein for replacement of wear and tear in the human body. The diet was planned, however, not to incorporate the barest minimum necessary to maintain nitrogen balance, but to allow a moderate surplus (or plus balance) of the standard protein so as to compensate for the minus balances which certainly would be encountered on cereal proteins, supplemented with only small quantities of milk and other proteins. To maintain a squad through 10

TABLE 2

Data on diet squad and average food consumption

	Height, cm.	*Weight, kg.*	*Surface area, sq.m.*	*Weight change, kg.*	*Caloric intake average*	*Prot. as per cent*	*C.H. of total*	*Fat calories*
V.C.[1]	175.5	75.3	1.91	—1.2	2865	5	44	51
J.H.	172.0	77.6	1.87	—4.5	2765	5	44	51
E.N.	171.5	70.8	1.83	—3.3	2815	5	43	52
W.P.	168.5	69.8	1.79	—3.9	2985	5	46	49
E.P.	171.5	61.8	1.74	—1.7	2665	5	45	50
O.S.	184.5	86.7	2.10	—4.9	3375	5	46	49
G.S.	172.0	71.1	1.84	—2.6	2760	5	44	51
M.S.[1]	174.0	72.2	1.86	—1.3	3300	5	44	51
C.S.	182.0	71.2	1.90	—1.1	2905	5	45	50
A.Y.	171.0	51.0	1.57	—1.3	2550	5	40	55
L.Y.	187.5	75.2	2.00	—3.9	3285	5	46	49
						5	44%	51

[1] M.S. replaced V.C. at end of ninth period.

weeks of extremely low nitrogen intake might be hazardous both to the health of the members and to the success of the experiment. The level chosen (average just under 6 gm. nitrogen, or about 37 gm. protein) was about as low as could be expected to maintain appetite and to avoid excessive losses of weight, even with all the vitamin requirements and the need for energy fully met. As it turned out, this average level of protein also was about as high as could be tolerated in the cereal periods. To get 6 gm. of nitrogen in the form of the inflated wheat, one must eat about one and one-half large

(3 ounce) packages. Even the granulated 'whole' wheat and wheat endosperm, which are among the least difficult to eat in large amount, tax a man's capacity pretty well up to the limit.

The protein content of the diet was kept constant at 5% of the total calories (table 2) and the carbohydrate and fat calories were kept close to 45 and 50%, respectively, after preliminary adjustments were made. Abundance of cream or butter was desired by all the squad to be eaten with the cereals. Excessive fat, however, is likely to be upsetting to some individuals. The success of the experiment proves that the levels of carbohydrate and fat were well selected, for only two members of the squad experienced nausea and this occurred only once for each. Only one subject exhibited diarrhea at any time, and this occurred only once. The diet naturally was not very filling so that the members of the squad frequently experienced hunger, but this insured good digestion and tolerance, and the subjects frequently remarked that they felt fine and slept well.

Vitamins were adequately supplied: A in cream, butter and egg; B in cereals supplemented by one vitamin B (complex) tablet [14] daily; ascorbic acid and citrin from fruits and fruit juices; D in egg; E in whole cereal, eggs, lettuce; riboflavin in egg, cream and fruits. Minerals also were well supplied: calcium in whole cereals and egg; phosphorus in the same; iron in eggs and lettuce.

Adjustments had to be made in the basal diet with every cereal used. Cornstarch bread [15] made into a small loaf with the help of gum acacia, hydrogenated cottonseed oil,[16] sugar and salt and baked in the oven supplied most of the carbohydrate in the egg periods; the balance was made up from sugar and applesauce. The excess of fat in egg was balanced in the cereal diet by adding to the butter and cream, both of which are quite compatible with the cereal diet.

[14] Harris'.

[15] The cornstarch bread used in this investigation differed from that reported in a previous publication (Sumner and Murlin, '38) chiefly in the substitution of sucrose for corn syrup and in the addition of cinnamon as flavor.

[16] Crisco.

The total calories in the two diets agreed perfectly, the distribution of calories was nearly equal in the two, and the distribution of protein between constituents other than egg and cereal was likewise quite satisfactory.

COLLECTION OF EXCRETA

The subjects were requested to report every slip, however slight, in collection of excreta. Seventy days for ten subjects makes a total of 700 urines and nearly as many stools. As might be expected in such a number, slight errors of collection occur. For example, one man dropped a urine bottle, breaking it and therefore lost 400 cc. of the day's urine, probably a third. Another lost 200 to 300 cc. of urine on 2 different days, and another passed a small amount of urine into another man's collection bottle. Another reports spilling of 25 to 30 cc. of urine. Two went through the entire 10 weeks without a slip of which they were aware. None of the accidents recorded was of so serious a nature as to cause rejection of the day, or to invalidate average results.

The alimentary rate on some of the cereals, and especially on the egg diet, was very slow, the marker (see below) not being found for as many as 4 days after it was eaten.

Urines were measured and analyzed for nitrogen every day. The feces were marked off with charcoal and collected in 5-day periods.

NITROGEN BALANCES

The values for the nitrogen balances of all the periods in the order of their occurrence are given in table 3. The order in which the cereals were eaten is of importance. Five subjects ate them in a given order, the other five in reverse order. It was expected that, descending from a relatively high protein intake to approximately one-third that ordinarily eaten, the subjects in the first cereal periods despite the preliminary period, would show minus N balances, but that toward the end of the series on the same cereal they would show some plus balances. This occurred as predicted, as

TABLE 3

Nitrogen balances (using average of last 3 days)

Subject	*1st egg*	*1st cereal Corn wheat*	*2nd egg*	*2nd cereal Toast wh. wheat*	*3rd egg*
V.C.	+0.50	—1.44	+0.74	—1.78	+0.70
J.H.	—0.07	—1.82	—1.19	—1.74	—0.11
E.P.	—0.04	—0.74	—0.30	—1.11	+0.38
A.Y.	—0.07	—1.77	+0.26	—1.30	+0.23
L.Y.	+0.06	—1.43	+0.32	—1.29	+0.71
Av.	+0.08	—1.45	—0.03	—1.44	+0.38
		Precooked oats		*Flaked wheat*	
E.N.	+0.50	—0.29	+0.49	—0.82	+1.75
W.P.	—0.87	—1.09	—0.39	—1.72	+0.70
O.S.	—0.36	—0.27	+0.01	—1.17	+1.08
G.S.	+1.13	+0.28	+1.24	—0.80	+1.59
C.S.	+0.12	—0.75	+0.51	—0.86	+0.05
Av.	+0.11	—0.45	+0.37	—1.07	+1.03
	3rd cereal Inf. wheat	*4th egg*	*4th cereal Wh. endosp.*	*5th egg*	*5th cereal Gran. wheat*
V.C.	—1.64	+0.63	M.S.—1.56	—0.18	—1.30
J.H.	—1.91	+0.01	—0.18	+0.63	—0.93
E.P.	—0.86	+0.54	+0.08	+0.60	—0.72[1]
A.Y.	—1.60	+0.47	—0.45	+0.57	—0.35[1]
L.Y.	—1.86	+1.00	—0.41	+0.84	+0.03[1]
Av.	—1.57	+0.53	—0.50	+0.49	—0.65
	Gran. wheat				*Inf. wheat*
E.N.	—0.14	+1.19	—1.25	+0.48	—1.28[1]
W.P.	—1.15	+0.79	—1.26	+0.09	—1.32[1]
O.S.	—0.14	+0.85	—0.34	+1.76	—1.34
G.S.	—0.48	+0.83	—1.01	+0.90	—1.15
C.S.	—0.45	+0.12	—0.46	+0.49	—0.78[1]
Av.	—0.79	+0.75	—0.86	+0.74	—1.17
	6th egg	*6th cereal Flaked wheat*	*7th egg*	*7th cereal Precook. oats*	*8th egg*
M.S.	—0.02	—0.30	+0.15	—0.29	+0.63
J.H.	+0.47[2]	—1.09	+0.16	—0.51	
E.P.	+1.08	—0.71	+0.46	+0.16	+1.18
A.Y.	+1.81	—0.85	+1.33	+0.22	
L.Y.	+2.01	—0.97	+0.68	+0.70	+1.08
Av.	+1.07	—0.78	+0.56	+0.06	+0.96
		Toast. wh. wheat		*Corn wheat*	
E.N.	+1.17	—0.72	+1.00	+0.07	+0.54
W.P.	+1.14	—0.43	+1.30	—0.39	+1.22
O.S.	+1.48	—0.56	+0.88	—0.20	
G.S.	+1.17	—0.66	+0.75	—0.70	
C.S.	+1.63	—0.44	+0.91	—0.59	+1.53
Av.	+1.32	—0.56	+0.97	—0.36	+1.10

[1] Calories increased 140 to 250, distribution kept same.

[2] Calories decreased 200, distribution kept same.

may be seen clearly in table 3. On torn wheat in the first cereal period all subjects were in minus balance, the average being —1.45 gm. In the last period the same cereal eaten in the same average amount shows all but one still in minus balance, but the average is now only —0.36. On oats only one was in plus balance the first time with an average of —0.45; in the last oats period three of the subjects were in plus balance and two minus, with an average now of +0.06. The same tendency can be seen in the toasted wheat-flaked wheat and in the inflated-granulated wheat periods. Each cereal when eaten by the second squad makes a better showing than when eaten by the first squad.[17]

This phenomenon clearly means that early in the series there was still some nitrogen coming from the surplus stores in the body and that late in the series these stores had been more nearly exhausted and the body consequently was laying claim to the ingested protein and retaining it for repair of the tissues with greater avidity.

Even in the egg periods the same tendency is evident. The individual plus balances become steadily more numerous up to the fourth time egg was eaten, when all become plus. This fact alone, the early arrival at plus balance for all, shows the superiority of egg protein over cereals generally. From the fourth to the seventh period there is only a little improvement with egg. The fourth and fifth periods, in fact, are identical for average values, and curiously enough stand just about as far on the plus side as wheat endosperm, between them, stands on the minus side.

The sixth egg period requires a word of comment. The average plus balance here is higher for both sub-squads than at any other time. The explanation lies in the fact that in the previous (or fifth) cereal period the calories were raised for six of the men because they were at this time losing weight a little too rapidly. The increase varied from 140

[17] This result is due in small part to the fact that six members of the squad had their calories increased in the fifth cereal period. However, one subject had calories decreased in the sixth egg period and three continued unchanged.

to 250 calories, and it is interesting that every man who got the increase shows a significantly better N-balance in the sixth than in the fifth egg period. The increased calories were derived about equally from carbohydrates and fat. Although the increased calories were continued to the end of the series the seventh egg protein doesn't maintain the same level as the sixth. Probably the return to the lower level of retention was due to a difference in external temperature. It was decidedly hotter during the 5 days of the sixth egg period than during the seventh. Calories not needed to maintain body temperature can be used to protect the protein.

TABLE 4

Comparative data on feces of all subjects for 5-day periods

	Torn wheat, gm.	*Toasted 'whole' wheat,* gm.	*Inflated wheat,* gm.	*Flaked wheat,* gm.	*Granulated wheat,* gm.	*Precooked oats,* gm.	*Wheat Endosperm,* gm.	*Egg* gm.
Weight	1009	772	682	630	600	562	340	315
Gm. N.	10.01	10.08	9.26	7.33	7.76	6.72	4.46	4.28
Per cent N	0.99	1.30	1.35	1.23	1.29	1.19	1.31	1.35

(In descending order of weight of stool.)

At first sight it would seem quite incorrect to raise the calorie intake in the midst of a long experiment. The higher intake, however, affects both egg periods and cereal periods from the fifth cereal to the end, and it is the difference in N-balance between the standard protein and the experimental one which determines replacement value.

NATURE OF THE FECES

The character of the stools naturally was affected by the amount of roughage contained in the cereals used. This was not unexpected, but the data were surprising. In table 4 are brought together the average weights of stools with their N content on the several cereals and on egg for all ten subjects for the 5-day period. Torn wheat gave the largest weight. On the average each man passed daily nearly ½ pound of feces on this diet. In practically all subjects the

stools were loose and a few could only be described as 'watery.' Fortunately they did not come at a time when the fecal marker was expected. The great weight of stool in fact was due in large part to water, but mixed with it were many strands of mucous, indicating abrasive effects on the intestinal lining. The relatively low nitrogen content of these stools naturally results from the high water content. Toasted 'whole' wheat came second, and lowest of all the cereals was wheat endosperm, because of its lack of roughage. Arranging the cereals in order of roughage places them also in nearly their order of stool production.

The coarser cereals eaten in the quantity required by this experiment were not well tolerated by some subjects. There were complaints of 'loss of strength' particularly on the torn wheat product. Wheat endosperm at the other end of the series on the contrary seems quite too bland in such large quantity. The volume of stool is so slight that it fails to arouse regularly the normal desire to defecate. The effect is the same as too much white bread in the diet. Nevertheless, many persons do best so far as regularity of bowels is concerned, on just this kind of bland, non-irritating diet. The pendulum apparently is swinging back from the emphasis on roughage of a few years ago.

It is an old dictum originating with the Voit school in Germany that "feces are essentially unabsorbed residues of the digestive juices"—not unabsorbed food primarily. It is generally known that feces do contain indigestible remnants of food such as berry seeds, apple peel, grains of corn, and so forth. But when one examines such a stool critically, it is found that the cause of bulk is not the included seeds, etc.; it is the matrix in which the seeds are embedded which is most increased. Hence the large stools from torn wheat are caused not so much by the addition of bran and fiber, as by the stimulation of secretion from the alimentary lining.

The proof of this statement lies in the figures for percentage of nitrogen in the stool. Except from those cereals which produce loosest stools (torn wheat, rolled oats) the percentage is very nearly the same. Wheat endosperm without any

bran produces just half as much stool as inflated wheat with all its bran (softened to be sure), but the percentage of nitrogen in them is practically identical. It is the same also as in stools from egg having no roughage whatever and from toasted 'whole' wheat, one of the most abrasive of cereals. Feces therefore come mainly from the same source, whatever the food.

This discussion of the nature of the feces is important for evaluation of the biological value of any given food protein. It has been a moot question for at least 75 years whether feces should be regarded as waste from the body or waste from food. Those who take the former view are supported by the present study. One cannot account in any other way for the closely similar nitrogen contents of stools from foods differing so much themselves in nitrogen content as inflated wheat (2.41%) and toasted 'whole' wheat (1.80%).

REPLACEMENT VALUES

In table 5 are listed the grand averages of ten subjects for the last 3 days on each diet. A question might reasonably be raised as to which egg period, the one which went before, or the one which followed each cereal, should be taken as standard. Using the average of the two enables one to compare each cereal protein with a high-value protein both in an earlier and a later stage of whatever change may be running progressively through the entire series. The line opposite 'egg' in table 5 contains these averages of both pre- and post-egg periods for the cereal which follows.

Observing the column under 'balance' it is seen that every cereal shows a negative balance. The range is from —0.10 gm. for oats to —1.28 gm. for inflated wheat. In contrast, every egg protein average shows a plus balance. The total range is only from +0.49 to +0.84 occurring in connection with the first two cereals. If all these plus balances were equal we should need no further calculation to arrive at the relative values of the several cereals. Their rating on the minus side alone would answer the question. But we have to consider the average nutritional condition of the several subjects

at the time (really just before and just after) the cereal was eaten. This information we get from the plus balances on egg.

Taking the difference between the average plus balances of the pre- and post-cereal egg periods on the one hand and the minus balance of the cereal period on the other, it is seen by how much the cereal fails of equality with egg. This figure alone would give us a rating of the cereals; but to get all on a comparable basis we take account of the small differences

TABLE 5

Replacement values of cereal proteins in terms of whole egg protein

	N output, grams per day			*Apparent digestibility*	*N intake gm./day*	*N balance*	*Egg N intake, gm./day*	*Replacement value, per cent*
	Urinary	Fecal	Total					
Egg[1]	4.49	0.97	5.46		5.95	+0.49	4.64	
Precooked oats	4.60	1.34	5.94	77	5.84	—0.10		87
Egg	4.40	0.71	5.11		5.94	+0.84	4.63	
Granulated wheat + wheat germ	4.98	1.62	6.60	73	6.04	—0.56		70
Egg	4.49	0.78	5.27		5.85	+0.58	4.56	
Wheat endosperm	5.66	0.89	6.55	85	5.87	—0.68		72
Egg	4.40	0.89	5.29		5.83	+0.54	4.55	
Torn wheat	4.74	2.00	6.74	66	5.84	—0.90		68
Egg	4.29	0.88	5.17		5.93	+0.76	4.63	
Flaked wheat	5.21	1.57	6.78	73	5.84	—0.93		63
Egg	4.36	0.87	5.23		5.89	+0.66	4.59	
Toasted 'whole' wheat	4.83	2.01	6.84	66	5.84	—1.00		64
Egg	4.38	0.81	5.17		5.88	+0.69	4.59	
Inflated wheat	5.37	1.85	7.22	69	5.94	—1.28		57

[1] Egg values are averages of pre-cereal and post-cereal periods for each subject.

in the egg nitrogen fed, and express the difference in balances as a percentage of egg protein. Thus, in the case of precooked oats 0.59 is 13% (nearly) short of egg; the cereal protein therefore rates 87% of perfect replacement of egg. How the other cereals rate is evident from the last column of figures.

Comparing fecal nitrogen for the different cereals, it is evident that wheat endosperm enjoys its high position in

replacement value because of the small wastage through the alimentary tract. Remembering what was said under 'nature of the feces' we may be reasonably certain that a great part of the loss in the feces from torn wheat and toasted 'whole' wheat is really loss from the body. Therefore wheat endosperm is digested and absorbed with least drainage of the body's resources in this respect. Replacing milk protein, Murlin and Mattill ('38) learned that wheat endosperm ranked 93% against milk, while rolled oats ranked 89 and granulated 'whole' wheat 83. Sumner, Pierce and Murlin ('38) report that wheat endosperm supplemented with one-tenth milk protein and one-tenth fruit protein replaced egg protein as well as milk, with a R.V. of 83. Precooked oats, however, in this study stands nearly the same as in the milk replacement study; granulated 'whole' wheat ranks considerably higher against milk than against egg, as does wheat endosperm. This looks like a decided difference between oats and wheat.

BIOLOGICAL VALUES

By assuming that egg protein has a biological value of 100 the cereals can be ranked in that scale. This expression of nutritional value in the narrower sense regards the fecal nitrogen, above a certain base value, not as waste from the body but as food residue. In table 6 it may be seen that egg fecal nitrogen, the base value, is subtracted from cereal fecal nitrogen (column a), and the remainder, regarded as fecal nitrogen from food, is subtracted from total food nitrogen (column d). This gives the so-called 'absorbed nitrogen' (column e). The value of the absorbed nitrogen is found as a difference between cereal urinary nitrogen (column f) and the basal egg urinary nitrogen (column g). The next column (h) shows that the average cereal nitrogen excretion for whole oats is 0.11 gm. more (called excess N) than the egg urinary output. This again is regarded as waste and when expressed as a percentage of the absorbed nitrogen amounts in the case of oats to 2.0%. Oats is found therefore to be 98% as good as egg, in its metabolic effect.

TABLE 6

Biological values

	a Cereal Fecal N, gm.	*b* Egg Fecal N, gm.	*c* (a–b) Fecal Food N, gm.	*d* Total Food N gm.	*e* (d–c) Absorbed N, gm.	True digestibility	*f* Cereal urin. N, gm.	*g* Egg urin. N, gm.	*h* (f–g) Excess N, gm.	*i* $\frac{Ex.\ N \times 100}{Abs.\ N}$ %	*j* B.V. 100–i %
Precooked oats	1.34	0.97	0.37	5.95	5.58	93.8	4.60	4.49	0.11	2.0	98.0
Granulated wheat + wheat germ	1.62	0.71	0.91	6.04	5.13	85.0	4.98	4.40	0.58	11.3	88.7
Wheat endosperm	0.89	0.73	0.16	5.87	5.71	97.2	5.66	4.49	1.17	20.3	79.7
Torn wheat	2.00	0.89	1.11	5.84	4.73	81.0	4.74	4.40	0.34	7.2	92.8
Flaked wheat	1.57	0.88	0.69	5.84	5.15	88.0	5.21	4.29	0.92	17.9	82.1
Toasted 'whole' wheat	2.01	0.87	1.14	5.84	4.70	80.5	4.83	4.36	0.44	10.0	90.0
Inflated wheat	1.85	0.81	1.04	5.94	4.90	82.5	5.37	4.38	0.99	20.2	79.8

By this method of reckoning, taking no account of differences in alimentary waste from the body, the cereals take quite a different rating. Torn wheat now stands second to oats, then toasted 'whole' wheat, and so on. Wheat endosperm ties with inflated wheat for the lowest place.

There is a serious objection to this method of calculating 'biological values' at least in the human subject. What is really in large part body waste is subtracted from the total fecal nitrogen to obtain the absorbed. Actually the absorbed nitrogen is considerably greater, but how much greater is not known. Many attempts have been made to separate food residues from the residues of the digestive secretions (see Murlin and Mattill, '38), but no satisfactory method has been found. The biological values for some cereals are too low because the absorbed nitrogen, the divisor in arriving at the percentage loss, is obviously too low. Thus the percentage excess loss comes out too high and subtracting from 100 leaves the biological value too low.

The two conceptions, the one we call egg replacement value of protein, the other biological value, represent extreme views neither of which is wholly correct. But replacement value at least takes account of both forms of waste. While we must admit that the fecal waste is not entirely from the body, certainly it is much more correctly so regarded than (all above the basal) as food waste.

It is interesting to note in table 5, that two of the whole wheats, flaked wheat and inflated wheat, yield a large urinary loss and large fecal losses as well. This gives them lower rank even in the biological value method (table 6) than the character of the grain with which they start would lead one to expect. They are but a shade better than wheat endosperm. Klein, Harrow, Pine and Funk ('26) showed that the fractions of the wheat berry which contain the pericarp and the germ possess a much higher nutritive value for the rat (B.V. by Mitchell's method) than fractions composed mainly of endosperm; thus, 'red dog' 76, standard middlings 64, patent white flour 53. This was confirmed by Boas-Fixsen and her

co-workers ('32, '34) who found that whole wheat gave a B.V. of 68, wheat embryo 69 and endosperm 61. With one exception (whole wheat) all these fractions by both groups of workers were studied at the same percentage level of intake (8%), and are therefore comparable. Mitchell's value (see summary table of biological values by Boas-Fixsen, '35) for whole wheat at the same level is 67. The two cereal foods giving values next higher than wheat endosperm in this study are supposedly entire wheat. The only reason which can be assigned for their lower rank than that of torn wheat and toasted whole wheat is the more severe heat treatment given them in the manufacturing process. The numerical values obviously are on a different level in our 'biological values' than in these just mentioned. We are dealing frankly with a combined or supplementary value of cereal nitrogen 78% with milk nitrogen 11% and fruit nitrogen 11%, in terms of the standard supplemented identically. These values therefore should be higher, as Mitchell and Carman ('26) found in rats. Reasons have been given in an earlier paper (Murlin and Mattill, '38) why this procedure is considered superior for man to the strict biological value procedure.

The heat injury effect of Morgan ('31) for whole wheat was 12, i.e., 64 to 52. The differences between torn wheat (92.8) on the one hand and flaked wheat (82.1) and inflated wheat (79.8) on the other, in this study are of the same order. It seems fair to infer that the lower values are due to heat injury. The manufacturers through their research departments have furnished information as to the temperatures reached and the exposure time (see p. 250). No other cereals in this study had been heated to such high temperatures. Comparison of true digestibilities proves that the heat injury to nutritive value concerns solely the effect on metabolism (table 6).

The heat injury reported by Morgan and Kern ('34) for meat autoclaved 1 hour at 15 pounds pressure, as compared with raw meat, is 11 B.V. units (67 to 56). The injury to peanut protein caused by roasting recently reported by

Mitchell and Beadles ('37) is much less, 2.1 B.V. units (57.9 to 55.8), difference in digestibility being slight. Seegers and Mattill ('35) have demonstrated a parallel lowering of digestibility and biological value in the case of liver heated above 100°C. However, Chick et al. ('36) have shown that heat injury to the B.V. of lactalbumen remains even when intake is adjusted to make allowance for decreased digestibility.

SUMMARY

1. A 10-week diet experiment on ten young men was carried out in 5-day periods in order to compare the nutritional value of the proteins of seven cereal breakfast foods with egg protein. Diets contained 5% of the calories in the form of protein, approximately 45% in the form of carbohydrate and 50% in the form of fat. Egg or cereal protein supplied 78% of the total nitrogen, cream and butter 11% and fruit and other accessory foods such as lettuce and a vitamin B complex tablet the remaining 11%.

2. Cereal protein alternated every 5 days with egg protein. One half of the diet squad ate the cereals in a certain order, the other half in reverse order. This served to balance whatever progressive change in the tendency to retain nitrogen might result from long-continued sustenance on low protein. The average level of nitrogen intake for all subjects was 5.92 gm., the egg period always equalling the following cereal period.

3. Replacement of egg protein by cereal protein is calculated by dividing the difference in nitrogen balance on the two diets by the amount of egg nitrogen fed. This difference expressed as a percentage is then subtracted from 100 to find the extent (in percentage) to which cereal replaces egg.

4. 'Biological value' also was calculated taking egg as standard. The difference between fecal nitrogen on cereal and egg is subtracted from food nitrogen to give 'absorbed nitrogen.' The difference between cereal and egg urinary nitrogen then is found and expressed as a percentage of the absorbed nitrogen. This value subtracted from 100 gives

(as a percentage) the extent to which the cereal protein absorbed replaces egg protein in the metabolism of the body.

5. A whole grain oats had the highest replacement and 'biological' values, 87 and 98, respectively. A wheat endosperm product gave the next highest R.V. (72) followed by a granulated 'whole wheat' supplemented with wheat germ (70), torn (shredded) whole wheat (68), toasted 'whole' wheat (64), flaked whole wheat (63) and inflated ('puffed') wheat (57). In the B.V. scale, oats was followed by the torn wheat (92.8), toasted 'whole' wheat (90), granulated wheat (88.7), flaked wheat (82.1), inflated wheat (79.8) and wheat endosperm (79.7).

6. The lower rank of the flaked and inflated whole wheats in both scales is believed to be accounted for by the high heat to which they are subjected in the process of manufacture. Digestibility was not diminished.

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MULTIPLE NATURE OF THE DEFICIENCY OF BLACKTONGUE-PRODUCING DIETS AS SHOWN BY STUDIES ON RATS

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ONE FIGURE

(Received for publication April 16, 1938)

It was the purpose of these investigations to study the effects of feeding rats blacktongue-producing diets. Although these diets are deficient in riboflavin (Birch, György and Harris, '35, and Richardson and Hogan, '36), chick antidermatitis (filtrate) factor (Jukes and Lepkovsky, '36 and Jukes, '37), as well as nicotinic acid (Elvehjem, Madden, Woolley and Strong, '37), the acute fatal conditions resulting from their ingestion by dogs, human beings, pigs and monkeys can be cured by administration of nicotinic acid alone (Elvehjem, Madden, Woolley and Strong, '37; Street and Cowgill, '37; Fouts, Helmer, Lepkovsky and Jukes, '37; Spies, Cooper and Blankenhorn, '37 and '38; Smith, Ruffin and Smith, '37; Chick, Macrae, Martin and Martin, '38; and Harris, '37 a, b).

Rats are known to require riboflavin (György, '35), chick antidermatitis (filtrate) factor (Lepkovsky, Jukes and Krause, '36), and nicotinic acid (György, '38) for satisfactory growth, yet normal or near normal growth of rats fed blacktongue-producing diets have been reported by Rhoads and Miller ('35), Birch, György and Harris ('35), Harris ('37 a) and Birch, Chick and Martin ('37). From the latter investigations it has been assumed that rats are less sensitive to the nutritional defect in diets which cause blacktongue than dogs, pigs, monkeys or human beings.

METHODS

The blacktongue-producing diet (Goldberger diet no. 195, Goldberger and Wheeler, '28) consists of corn, whole white ground, 400 gm.; cowpeas ground, 50 gm.; casein purified, 60 gm.; sucrose, 32 gm.; cottonseed oil, 30 gm.; cod liver oil, 15 cc.; and salt mixture (Osborn and Mendel, '13) 22 gm. The rats were maintained in separate, double-bottom wire-mesh cages. Supplements were fed from glass coasters. Rats were weighed three times weekly. A portion of the rats was fed for periods of 2 weeks on vitamin G(B_2) free diet before receiving blacktongue-producing diet. The majority of rats received blacktongue-producing diet from time of weaning. No difference was noted.

The daily supplements used consisted of powdered liver extract, Lilly (200 gm.); crystalline riboflavin (40 micrograms); purified liver extract (0.05 cc.) containing chick antidermatitis factor and nicotinic acid but free from rat antidermatitis factor and riboflavin; and nicotinic acid (1.0 mg.). The purified liver extract was supplied by Dr. Samuel Lepkovsky.

RESULTS

Studies extending over 2½ years indicate that subnormal growth rates result from feeding rats unsupplemented blacktongue-producing diets. The first group of rats survived for many months (maximum 9 months). Rate of growth averaged 12.7 gm. per week during the first 4 weeks on the diet. Weight became stationary at between 170 and 230 gm. Resumption of growth did not follow addition to the diets of purified liver extract containing chick antidermatitis factor and nicotinic acid, or of vitamin B_6, the rat antidermatitis factor. However, if riboflavin was added, even after periods of 230 days, there was an increase in the rate of growth (fig. 1). This increase was not sustained in some rats, but substitution of powdered liver extract for riboflavin in these animals was followed by further weight gain. Animals showing rapid weight loss after several months on the diet did not respond to riboflavin and occasionally not to powdered liver extract.

The response of these rats to riboflavin suggested that the natural foodstuffs in the diet supplied sufficient amounts of other components of vitamin G(B_2) complex for growth or that the rats' reserve stores of riboflavin were more quickly depleted than the stores of other components of the complex.

In repeating the above experiments it was found that rate of growth of all rats on unsupplemented diets was even less than that in the first experiment. Many showed little or no gain in weight. Average weekly gain in weight of fifty-five rats was only 7.8 gm. during the first 4 weeks on diet.

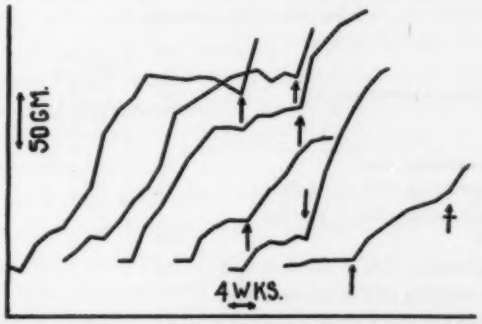

Fig. 1 Weight curves of rats in first group studied on unsupplemented black-tongue-producing diets. Arrows indicate administration of riboflavin; crossed arrow indicates administration of powdered liver extract.

Addition of riboflavin produced no appreciable increase in rate of growth. Variation in storage of components of vitamin G(B_2) in rats or differences in vitamin content of natural foodstuffs in diets could account for this variation in response.

Only rats receiving no supplements or supplements from beginning of study were included in table 1. Adequate growth did not occur even when the diet was supplemented with riboflavin (40 micrograms), with purified liver extract (0.05 cc.), with nicotinic acid (1.0 mg.) or with both riboflavin and nicotinic acid. Rats receiving both riboflavin and nicotinic acid, however, showed better growth rate than those

receiving either separately. Rats on diets supplemented only with nicotinic acid gained even less weight than those on the unsupplemented diets.

Satisfactory growth was obtained when rats were fed blacktongue-producing diets supplemented daily with powdered liver extract (200 mg.), or with riboflavin (40 micrograms) and purified liver extract (0.05 cc.). The latter combination of supplements with added nicotinic acid (1.0 mg.) was

TABLE 1

Average gain in weight of rats on blacktongue-producing diet and blacktongue-producing diet supplemented with various components of vitamin G(B_2) complex

DIET AND SUPPLEMENTS	NUMBER OF ANIMALS	AVERAGE GAIN PER WEEK; FIRST 4 WEEKS, IN GRAMS	NUMBER OF ANIMALS	AVERAGE GAIN PER WEEK; FIRST 6 WEEKS, IN GRAMS
Blacktongue-producing diet	55	7.8	44	7.4
Blacktongue-producing diet + riboflavin	23	7.7	11	7.9
Blacktongue-producing diet + purified liver extract	10	10.3	3	10.1
Blacktongue-producing diet + nicotinic acid	14	5.5	12	5.3
Blacktongue-producing diet + nicotinic acid and riboflavin	17	9.2	17	9.2
Blacktongue-producing diet + purified liver extract and riboflavin	25	16.4	18	16.3
Blacktongue-producing diet + nicotinic acid, purified liver extract and riboflavin	7	21.2	7	19.5
Blacktongue-producing diet + powdered liver extract	17	18.3	16	18.4

followed by the most rapid gain in weight. This suggests that the purified liver extract did not supply sufficient nicotinic acid in the amounts fed.

These studies demonstrate the multiple nature of the deficiency of blacktongue-producing diets. They also confirm the previous finding that rats are not as sensitive to deficiency of nicotinic acid as dogs, monkeys or pigs. Nicotinic acid alone will cure the acute fatal conditions resulting from ingestion of blacktongue-producing diets by these latter animals.

This does not necessarily indicate that in long-term experiments nicotinic acid alone will completely supplement diets for these animals. Sebrell, Onstott and Hunt ('37) have found that dogs on a blacktongue-producing diet to which substances containing blacktongue-preventive factor are added will die unless riboflavin is also present.

In long-term experiments many rats died when the diet was not properly supplemented. Only two rats receiving powdered liver extract or the combination of riboflavin and purified liver extract died during the period of study. The highest mortality was noted in rats receiving diets supplemented only with nicotinic acid. This high mortality in long-term experiments and decrease in growth in rats receiving the blacktongue-producing diets supplemented only with nicotinic acid demonstrates the possible harmful effects of administration of one component of vitamin G(B_2) complex in absence of the other components.

The rats' coats appeared normal even when growth was poor, but in some animals they become discolored. A few animals in the long-term experiments showed loss of hair. This condition disappeared when riboflavin was added to the diet. Dermatitis about the eyes occurred rarely. There was no definite symptom complex noted in absence of proper supplements. The outstanding abnormality was failure of proper growth. Often after many months on the diets, sudden rapid decrease in weight occurred, and these rats died unless the diets were supplemented with powdered liver extract although this did not always save their lives.

CONCLUSIONS

1. Blacktongue-producing diets did not contain sufficient riboflavin, chick antidermatitis (filtrate) factor, or nicotinic acid to promote normal growth of rats.

2. Addition of riboflavin to the diet increased the growth rate in one experiment but not in the others.

3. Rats fed diets supplemented only with nicotinic acid grew less than rats fed unsupplemented diets.

4. Since blacktongue-producing diets are similar to diets of pellagrous patients, these findings suggest that if nicotinic acid alone is added to diets of pellagrins, deficiencies due to other missing components of vitamin G(B_2) complex may develop. It is suggested that treatment of pellagrous patients should include administration of all known components of the complex even though it has been reported that administration of nicotinic acid alone cures pellagra.

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THE EFFECT OF RIBOFLAVIN ON THE INCIDENCE OF CURLED TOE PARALYSIS IN CHICKS

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(Received for publication March 23, 1938)

Several investigators have reported the occurrence of a nutritional paralysis in chicks on rations low in the vitamin G complex. Norris, Heuser and Wilgus ('30) and Norris, Heuser, Wilgus and Ringrose ('31) first observed and described the syndrome, which they found could be cured by a concentrate from milk. Bethke, Record and Kennard ('31) also observed the disorder and found that it could be prevented by dried skim milk, dried whey, a concentrate from milk, alfalfa leaf meal, autoclaved yeast and liver meal. Later Bethke, Record and Wilder ('37) reported that pure lactoflavin would prevent the leg disorder. Norris, Wilgus, Ringrose, Heiman and Heuser ('36) suggest that the nutritional paralysis is due to a deficiency of some other factor rather than to a deficiency of the growth promoting vitamin G. They observed that the addition of small quantities of vitamin G supplement to the basal diet often increased the amount of this disorder, whereas the addition of large amounts decreased it.

Norris et al. ('36) designate this syndrome as nutritional paralysis, while Bethke et al. ('37) call it a leg disorder. As other workers, Elvehjem, Phillips and Hart ('37) have described paralysis of dietary origin in chicks, not identical with that produced by a deficiency of vitamin G, it would be desirable to designate the paralysis due to vitamin G deficiency by a more specific name. The name curled toe paralysis will be used through the remainder of this discussion, since as Norris

et al. ('31) have shown, it characteristically describes the manner in which the toes curl in and under in this deficiency disease.

The observations reported here were made during experiments with pure riboflavin supplements. They are in conformation with the observations of Norris et al. ('36) that the addition of small amounts of vitamin G supplement increased the severity of the disorder, as it was found that small amounts of riboflavin greatly increased the incidence of curled toe paralysis, while larger amounts completely prevented it.

EXPERIMENTAL

Basal ration 2, having the following composition was used throughout: 20% water washed fish meal, 40% yellow corn, 20% wheat middlings, 3% rice bran filtrate,[1] 3% soybean oil, 1% sodium chloride, 12.5% cornstarch and 0.5% high potency sardine oil[2] (400 units vitamin D and 3000 units vitamin A per gram). The washed fish meal was prepared by washing a commercial sardine meal ten times with about 15 volumes of hot water (60° to 70°C.) and then drying in a vacuum oven. Day-old White Leghorn chicks were used throughout, being maintained in electrically heated batteries and given feed and water ad libitum. The riboflavin used in experiments 3 and 11 was a crystalline preparation.[3] Its purity was 50% as determined in a photoelectric colorimeter, by the method of Norris and Sullivan (personal communication). The riboflavin used in experiment 6 was a solution of pure crystals (PX grade) from the Borden Company. The levels of riboflavin fed represent the pure material and are based on the results of colorimetric determinations. The whey adsorbate used contained 80 micrograms of riboflavin per gram, as determined by a biological assay with chicks. The supplements added replaced an equal amount of starch.

[1] Supplied through the courtesy of the Vitab Products, Inc., Emeryville, California.

[2] Supplied through the courtesy of the F. E. Booth Co., Inc., San Francisco, California.

[3] Supplied through the courtesy of Dr. S. Lepkovsky.

Experiments 3 and 6 consisted in depleting chicks for 2 weeks on a riboflavin free diet, then feeding the riboflavin supplements for 2 weeks. The severity of curled toe paralysis was designated in the following way:

+, tendency to rest on hocks and slight curling of toes.
++, marked weakness of legs and distinct curling of toes of one or both feet.
+++, toes completely curled in or under. Legs weakened so that bird continually walks on hocks.

The curled toe paralysis score represents the average number of plus signs per chick on the final day of the experiment.

TABLE 1

Experiments 3 and 6. Effect of graded amounts of riboflavin on curled toe paralysis

Experiment no.	*Supplement per 100 gm. of diet*	*Weight at 28 days*	*Average curled toe paralysis score at 28 days*	*Per cent of chicks affected at twenty-eighth day*
		gm.		
3	None	73.7	0.00	0
	60 micrograms riboflavin	102.0	0.81	44
	117 micrograms riboflavin	146.0	0.89	38
	292 micrograms riboflavin	183.5	0.00	0
	5 gm. whey adsorbate	190.9	0.00	0
6	None	83.3	0.00	0
	53 micrograms riboflavin	94.1	0.36	18
	106 micrograms riboflavin	122.0	0.72	36
	160 micrograms riboflavin	140.3	0.87	53
	266 micrograms riboflavin	198.4	0.07	7
	532 micrograms riboflavin	202.5	0.00	0
	1 gm. whey adsorbate	107.0	1.07	53
	2 gm. whey adsorbate	153.2	0.87	47
	3.5 gm. whey adsorbate	191.6	0.00	0
	5 gm. whey adsorbate	198.0	0.00	0

The results of experiments 3 and 6, presented in table 1, show that in the absence of added riboflavin no cases of curled toe paralysis had appeared by the end of 28 days. Addition of suboptimal amounts of riboflavin induced the disorder in both experiments, while larger amounts, adequate to permit normal growth, completely prevented it. The effect of adding varying amounts of whey adsorbate was similar to that produced by adding the pure riboflavin.

As many of the experiments reported by Bethke et al. ('37) were of 8 weeks duration and the supplements were given from hatching time, another experiment was conducted using a 6-week test period, without a previous depletion period. The results of this experiment, presented in table 2, confirm the results of the previous experiments in that small amounts of riboflavin increased the incidence of curled toe paralysis and larger amounts decreased it.

TABLE 2

Experiment 11. Influence of riboflavin on incidence of curled toe paralysis

Supplement per 100 gm. of diet	*Weight at 42 days*	*Average curled toe paralysis score at 42 days*	*Total per cent of chicks affected*
None	82.8	0.06	6[1]
83 micrograms riboflavin	170.7	1.13	47
163 micrograms riboflavin	327.4	0.13	6
245 micrograms riboflavin	366.3	0.00	0
370 micrograms riboflavin	407.3	0.00	0
490 micrograms riboflavin	389.1	0.00	0
5 gm. whey adsorbate	417.8	0.00	0

[1] Only 25% of the chicks were alive on the forty-second day. The average score and per cent chicks affected is based on the number of chicks started.

This same effect has been repeatedly observed. Very few cases of curled toe paralysis have been observed on the unsupplemented basal ration, while with suboptimal amounts of riboflavin supplements, many cases have appeared.

DISCUSSION

It must be noted that, while we found very few cases of curled toe paralysis where no riboflavin supplements were given, Bethke et al. ('37) observed a 35% to 65% incidence on diets containing washed or unwashed Argentine casein or Labco casein,[4] without the further addition of small amounts of riboflavin supplements. The appearance of paralysis on these rations may be due to the fact that they supported more growth and thus probably contained more riboflavin than the rations used here. The weights of chicks at 8 weeks of age

[4] Manufactured by the Casein Company of America.

reported by Bethke et al. ('37) were 161, 152 and 151 gm. for unwashed and washed Argentine and for Labco caseins respectively, while the chicks on basal ration 2 weighed only 83 gm. at 6 weeks of age. As 75% had died before the end of the sixth week and the survivors were gaining at the rate of a few grams a week, it is improbable that they would have weighed more than 90 gm. at 8 weeks. This indicates that the ration used here was probably lower in riboflavin than that used by Bethke et al. ('37). As washed casein should be low in riboflavin, the difference may be due to locational variation in the riboflavin content of the cereals.

CONCLUSION

1. The effect on curled toe paralysis of adding graded amounts of pure riboflavin to a diet low in that factor has been studied.

2. Under the conditions of the experiments, curled toe paralysis did not develop unless small amounts of riboflavin were present. Large amounts of the factor completely prevented the disorder.

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FATTY LIVERS IN VITAMIN B_6 DEFICIENT RATS

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(Received for publication February 28, 1938)

During the course of experiments carried out at the University of California (Halliday and Evans, '37 a, b, c) it was noted, although not reported, that the majority of the vitamin B_6 deficient rats which were autopsied showed enlarged yellow livers. Since this usually indicates a fatty 'degeneration' it was suspected that the carbohydrate and fat metabolism of the animals was abnormal. The basal diet contained 73% sucrose and only 5% fat.

The production of fatty livers may be due to any one of a number of causes. The entire field is too extensive and complicated to be reviewed at this time. However, the work of McHenry ('35, '36, '37 a, b, c) and his study of the interrelationship between choline and vitamin B_1 with respect to fatty livers in rats was considered to be of particular interest.

The present experiments were planned to study the relationship, if any, between vitamin B_6 and fatty livers. Choline was given to some of the rats to determine whether the high degree of purity of the basal diet led to a deficiency in this essential material. It was originally planned to give to some of the rats a highly purified concentrate of vitamin B_6 to determine its value in preventing or curing the fatty livers. However, due to a change in position of the author, this phase of the problem could not be carried out.

Most of the liver extracts were analyzed while perfectly fresh, but a period of about 3 months elapsed in two cases

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(groups 8B and 3A, table 1) between the preparation of the liver extracts and the analysis. Because of this most of the extracts were subjected to a second analysis to determine the extent of losses on standing.

EXPERIMENTAL

In order to determine the normal values for rats in this laboratory the livers from several groups of rats of ages varying from 23 days to 1 year were analyzed. In the case of the young rats (and in all cases of experimental animals) the livers were too small to allow for complete analyses, and several were combined for extraction. The animals were anesthetized and the livers removed before death. The Bloor technics ('28, '29, '37) for extraction and analysis of the lipids, and the Yasuda ('31) method for iodine number were followed. Determinations were made of total fatty acids, phospholipid fatty acids, total cholesterol and iodine number.

The experimental animals were weaned at 25 to 28 days of age, with a body weight of approximately 40 gm. They were killed after varying lengths of time on the experimental diet. All rats received 10 γ of vitamin B_1[2] and 20 γ of riboflavin [2] daily from weaning as a separate supplement. When choline [2] was fed, a solution of the material was added to the vitamin supplement. One group (5B) received a total of 40 mg. of choline in 4 days, and the second received 220 mg. in 11 days (6B). The basal diet was identical, as far as possible, with that used in the California laboratory.

Rats in this laboratory developed vitamin B_6 deficiency rather more slowly than did those in California. For this reason one litter, with the mother, was given the deficient diet for the final week before weaning (group 8B).

[2] The following materials were generously contributed: vitamin B_1 crystals from Merck and Company, Inc., through the courtesy of Dr. R. E. Gruber; riboflavin by Vitab Products, Inc., through the courtesy of Mr. G. F. Siemers, and also from Dr. S. Lepkovsky of the University of California; and choline by Dr. E. W. McHenry.

RESULTS

The results obtained are shown in table 1.

Although the experiment is incomplete there are a number of points of interest in the results.

When expressed as milligrams per gram of body tissue it is seen that the weights of the livers of the normal animals were very uniform, regardless of the weight or age of the rats. The livers of the deficient rats weighed one-third to one-half higher; while those of the choline-supplemented rats fell between the other two values.

The total fatty acid contents of the livers of the normal rats were about one-half to one-third as high as those of the deficient rats. There appears to be a tendency to a drop in the fatty acid content with age. Even massive doses of choline failed to bring the level of liver fatty acids in the choline-fed rats to normal. (The 60-day-old normal rats showed a total fatty acid value of about 25 mg. per gram of liver tissue, while the choline-fed and deficient rats contained 37 and 70 mg. per gram of tissue, respectively.)

As was to be expected, the phospholipid fatty acid content of the livers of all animals was essentially the same.

There was also practically no difference in the cholesterol values between the groups, although there appeared to be a slight drop in the livers of the older animals.

Considerable variation was found in the iodine numbers. One interesting point is that high iodine numbers are found in livers which contained the highest percentage of fatty acids and is a finding which is unusual. Slightly lower values were found in the livers of the more mature rats.

EFFECT OF STORAGE

The alcohol-ether extracts of the livers were kept in clear glass bottles at room temperature partially shielded from light. A period of 4 months elapsed between the first and second analyses of extracts 2A, 4A, 1B and 2B; extracts 4B, 5B, 6B and 7B were stored approximately 3 months between the two sets of analyses, and extracts 3A and 8B were stored for 3 months before any analysis was carried out.

TABLE 1

Results of analyses of livers from normal and vitamin B_6-deficient rats and deficient rats which had received choline

GROUP	NUMBER OF RATS	AGE	AVERAGE WEIGHT OF RATS	WEIGHT OF LIVER PER GRAM RAT	TOTAL FATTY ACIDS, MILLIGRAMS PER GRAM LIVER		PER CENT LOSS	PHOSPHOLIPID FATTY ACIDS, MILLIGRAMS PER GRAM LIVER		PER CENT LOSS	CHOLESTEROL, MILLIGRAMS PER GRAM LIVER		IODINE NO.		PER CENT LOSS
					Analysis			Analysis			Analysis		Analysis		
					1st	2nd		1st	2nd		1st	2nd	1st	2nd	
Normal		*days*	*gm.*	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*			
1A	9	23	40	40.8	33.1	...	..	27.5	...	..	2.1	..	119	...	..
2A	4	25	44	35.4	34.6	31.8	8	27.8	22.3	20	2.0	2.1	124	111	10
3A	4	25	41	41.0	...	30.5	..	...	22.8	..	..	1.9	...	102	..
4A	2	60	120	40.0	24.6	22.8	8	26.6	19.6	27	1.2	1.2	102	85	16
5A	1	1 year	300	40.5	28.0	...	..	24.0	...	..	1.6	..	83	...	..
B_6-deficient															
1B	4	61	44	59.0	78.0	68.4	12	22.2	16.5	26	3.0	2.4	135	110	18
2B	3	58	48	47.0	75.8	63.4	16	26.0	19.7	24	2.1	2.0	122	106	12
4B[1]	5	66	47	53.0	66.2	57.5	13	23.4	19.2	18	2.0	2.0	127	106	17
7B[1]	7	61	46	54.4	67.7	55.0	17	25.1	20.0	20	2.6	2.1	122	102	16
8B[2]	4	41	41	54.5	...	56.6	..	...	19.0	..	..	2.2	...	98	..
B_6-deficient + choline															
5B (40 mg.)	6	66	47	44.2	37.0	35.2	5	24.0	20.2	17	2.0	2.0	109	98	10
6B (220 mg.)	8	61	46	46.0	38.6	36.6	5	25.2	20.6	18	2.6	2.0	112	100	11

[1] Groups 4B and 5B; 6B and 7B are litter mates.

[2] 8B—mother and young given deficient diet 1 week before weaning.

Except for cholesterol values there was a loss of all lipids. Since ester and free cholesterol determinations were not made, it is not possible to state whether hydrolysis of the ester had occurred. The greatest loss of total fatty acids, both real and as per cent of total, was found in the extracts of livers from the deficient animals. This may be due to a difference in the nature of the fatty acids, since the iodine numbers of these extracts were the highest of the group. There was a large and fairly regular loss in the phospholipid fatty acids in all extracts. The iodine numbers, as was to be expected, dropped considerably, probably due to oxidation.

Boyd ('37) has recently reported a study of the losses found to occur in an alcohol-ether extract of plasma on storage. He found a significant decrease in phospholipids between 1 and 3 months, and between 3 and 6 months there occurred a significant decrease in cholesterol esters, total cholesterol and total lipid, a significant increase in neutral fat and free cholesterol, and no appreciable change at any time in total fatty acids. The present results differ markedly from his in respect to total fatty acids, particularly in the case of the extracts of livers from deficient animals. Because of the drop in iodine numbers it might seem that oxidation of the fatty acids had occurred and that the loss was not compensated for by hydrolysis of cholesterol esters and phospholipid fatty acids.

SUMMARY

A study was made of the lipid content of livers of rats deficient in vitamin B_6, as compared with normal animals and with deficient animals which had been given choline.

The results show a significantly heavier liver containing a higher percentage of total fatty acids in the deficient animals. The addition of choline remedied the condition to a large extent, but even massive doses failed to bring the liver weight and total fatty acid content quite to normal.

Storage of the lipid extracts for a period of about 3 months resulted in losses in both total fatty acids and phospholipid fatty acids, no change in total cholesterol values and a definite drop in all iodine numbers.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the helpful interest and advice of Prof. W. R. Bloor throughout the course of these experiments.

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THE EFFECT OF ROUGHAGE ON THE CALCIUM BALANCE IN RATS[1]

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(Received for publication March 9, 1938)

There has been a general belief that a large bulk of indigestible food material, particularly of fiber and cellulose residues, in the alimentary tract lowers the utilization of important nutrients. However, the evidence supporting this belief is not clear-cut.

In the case of calcium the evidence on the effect of roughage is confusing. Voit (1880) reported a loss of calcium through excessive production of feces and Sjollema ('23) observed that an increase in indigestible fiber invariably causes a higher fecal output of calcium. It is important to note, however, that, in experiments involving calcium of vegetable origin, rather complex food materials have often been used, in which part of the ingested calcium is associated with food materials which are themselves poorly digested. On the other hand, Blatherwick and Long ('22), Mallon, Johnson and Darby ('33), Fincke and Sherman ('35) and others have showed that lowered retentions are not necessarily to be expected with calcium from vegetable sources. Ascham ('31), using cellulose flour with dogs, and Morgan ('34), employing regenerated cellulose with human subjects, reported that an addition of roughage to the diet is accompanied by an increased calcium elimination in the feces. Such an effect of roughage

[1] Aided by grants from the Rockefeller Foundation and from the Research Fund of the School of Medicine, Yale University.

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was not confirmed by Bloom ('30) who used filter paper at an 8% intake level. Aub, Tibbetts and McLean ('37) assert that fecal volume has practically no effect on the fecal calcium.

A high roughage content is characteristic of the Chinese rural diet and the intake of calcium also is low, the figures reported for the latter being generally below the accepted standards. It is, therefore, of great practical importance to determine whether the presence of a large amount of fiber and cellulose in the diet impairs the utilization of calcium which is already at a suboptimal level.

The experiments here reported were carried out with rats to examine the effect of diets containing an exaggerated amount of roughage in the form of regenerated cellulose [3] on the calcium balance. Measurements were likewise included on a low calcium diet with and without roughage, for the purpose of estimating the so-called metabolic calcium under these same conditions.

EXPERIMENTAL

Diets were prepared as indicated (table 1) and litter-mate rats were divided into two groups; one was given a control diet and the other, a cellulose-rich diet. The sexes were also evenly divided. Care was taken to use materials for the basic diet which were practically calcium free. The Wesson ('32) salt mixture was used but was altered so as to omit all calcium while including the usual amount of phosphorus. Calcium lactate was added to diets 1 and 2 in amounts sufficient to provide an intake of 40 to 50 mg. of calcium per rat per day with a Ca-P ratio 1.2:1. The cellulose used was practically calcium free. Because of the difficulty in grinding this material alone, a cellulose-starch mixture was first prepared. An amount of starch was treated with water on the steam bath to make a thick paste; to this an equal weight of the regenerated cellulose was added and the whole thoroughly mixed and dried. The resulting product, cellulose-starch (1:1), was easily reduced to a powder in the grinder. The roughage

[3] Regenerated cellulose, supplied by the Sylphrap Corporation.

diets, diets 2 and 4, were identical with diets 1 and 3 except that they contained in each case 25% of cellulose. Another dietary series planned to use cellulose flour as a source of cellulose was abandoned because it was found the cellulose flour itself contained an appreciable amount of calcium.

The rats were placed in individual metabolism cages patterned after the type used by Ashworth ('35) which permitted a very precise separation of urine and feces. Each cage consisted of a 4-liter beaker; the ½-inch gauze on which the rat

TABLE 1

Percentage composition of the diets fed

		Diet 1	*Diet 2*	*Diet 3*	*Diet 4*
Starch		32.5	25.0	32.5	25.0
Cellulose		0.0	25.0	0.0	25.0
Lactalbumin [1]		20.0	15.0	20.0	15.0
Crisco		31.0	23.4	31.0	23.4
Yeast		5.0	3.7	5.0	3.7
Cod liver oil		5.0	3.7	5.0	3.7
Salt mixture [2]		2.7	2.0	2.7	2.0
Ca lacate		3.77	2.83	0.0	0.0
Ca content (%)	Series 1	0.528	0.395	....	....
	Series 2	0.532	0.399	0.019	0.024
P content (%)	Series 1	0.469	0.341	....	....
	Series 2	0.444	0.333	0.333	0.259

[1] Dry Milk Co., Inc.: Lactalbumin 7HAA.

[2]

KH_2PO_4	376	NaF	0.57
NaH_2PO_4	75	$CuSO_4 \cdot 5H_2O$	0.39
NaCl	105	$MnSO_4$	0.20
KCl	120	$KA1(SO_4)_2 \cdot 12H_2O$	0.09
$MgSO_4$	90	KI	0.05
$FeSO_4 \cdot 7H_2O$	18		

rested and the other metal parts at the bottom of the cage were of aluminum and galvanized iron with glass rod supports. The food cup was suspended from the top of the cage, the food in all cases being of a pasty consistency to avoid scattering. A 10-day metabolism period was employed, composed of a preliminary period of 4 days followed by a collection period of 6 days; excreta were analyzed separately for each rat. Experience had indicated that 4 days was sufficient time for the rat to adjust to a new diet in this type of experiment.

The rats were fed on the paired feeding plan; preliminary trials had indicated the amount which the animals would consume daily, and this was increased slightly as the body weight increased. The amounts fed to the two groups were

TABLE 2

Effect of roughage (cellulose) on calcium and phosphorus balance: calcium-rich diet averaged totals for 6-day periods, figures in milligrams

	Number of rats	Average weight, gm.	Ca intake	Urinary Ca	Fecal Ca	Ca balance	P intake	Urinary P	Fecal P	P balance
Series 1:										
Diet 1 (control)	6	300	356	4.4	192	160	316	157	66	93
Diet 2 (cellulose)	6	306	347	3.9	196	147	301	126	95	80
Series 2:										
Diet 1 (control)	8	98	225	2.8	76	146	187	64	33	90
Diet 2 (cellulose)	8	99	223	3.1	87	133	186	65	49	72

TABLE 3

Effect of roughage (cellulose) on calcium and phosphorus balance: calcium-poor diet averaged totals for 6-day periods, figures in milligrams

	Number of rats	Average weight, gm.	Ca intake	Urinary Ca	Fecal Ca	Ca balance	P intake	Urinary P	Fecal P	P balance
Series 2:										
Diet 3 (control)	8	166	8.1	1.8	8.7	−2.4	141	120	13	+8
Diet 4 (cellulose)	6	170	12.0	1.2	6.7	+4.1	148	113	39	−4

identical on the basis of non-roughage content; the experimental group consumed, for example, 12 gm. of the roughage-containing diet, while the control group consumed but 9 gm.

Two series of experiments were carried out, one in America, one in China, using similar diets and following identical procedures. Balance experiments on the low calcium diet

were included in series 2 only. All the determinations were made by micro methods using the method of Kramer and Tisdall ('21) for calcium and that of Fiske and Subbarow ('25) for phosphorus. The balance data, summarized for each group, are shown in tables 2 and 3.

RESULTS AND DISCUSSION

The balance experiments here reported show a very slight increase in excretion of fecal calcium with the cellulose diet. This would confirm the finding of Ascham ('31) who reported that with dogs roughage tends to increase the fecal calcium. This effect of roughage may be regarded, however, as of no practical significance, for even with the large quantities of roughage fed, 25% of the total intake, the calcium loss is less than 10% of the calcium retention. It is still less significant when compared to the total amount of the calcium intake.

Very low figures are obtained for calcium excretion in the urine on all diets, and the amount is as low as 0.2 mg. per rat per day on the low calcium diet. These low figures for urinary calcium testify to the efficiency of the arrangement used for separating urine and feces.

The experiment on the low calcium diet with and without cellulose was included for the purpose of determining whether a large bulk of roughage tends to cause a pronounced excretion of calcium of metabolic origin, similar to the increase in metabolic nitrogen which has been demonstrated for nitrogen-free roughage-containing diets (Mitchell, '24). It is conceivable that a large alimentary bulk might possibly stimulate an increased secretion of bile and other calcium-containing digestive adjuncts. The results on the calcium-poor diet recorded in table 3 indicate, however, that this is not the case; there is no increase in excretion of metabolic calcium. It is conceivable that roughage in the intestinal tract may either interfere with absorption, or increase the calcium excretion through the intestine. It may be inferred from the experiments here reported that the former supposition explains best the slight increase in fecal calcium. It may also

be noted that this increase in fecal calcium is accompanied in each case by an approximately equivalent increase in fecal phosphorus.

We were unable to secure regenerated cellulose of lower calcium content than that used in this experiment. The material used contained only about 0.03% of calcium, but even this amount was sufficient to change the negative calcium balance on the calcium-poor diet (table 3) into a positive one.

Studies on roughage-containing diets have often confused effects which may be due to 1) the use of nutrients intrinsically low in digestibility; 2) the presence of substances which specifically cause laxative action and watery stools; and 3) a large alimentary fill of non-digestible, inert material. Laxatives merely tend to hasten the passage of material through the alimentary canal so that the nutrients are not all absorbed. With respect to inert alimentary fill, Adolph and Wu ('34) have shown in experiments with rats, where clay constituted as much as 60% of the diet fed, that there was no significant lowering of the digestibility of the protein fraction of the diet.

Our experience with regenerated cellulose indicates that, while it furnishes the desired bulk, it is chemically quite inert and does not easily absorb water. The feces from the animals on the cellulose diet in these experiments were in all cases well formed and firm. There were no cases of soft watery feces such as are found when large amounts of agar agar are incorporated in the diet.

SUMMARY

Balance experiments show that when regenerated cellulose is fed as roughage to rats on a 25% intake level, there is no significant increase in the fecal output of calcium and phosphorus. When rats are fed a diet practically calcium free to which regenerated cellulose has been added, there is no evidence of an increased calcium excretion.

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A COMPARISON OF FERROUS AND FERRIC IRON IN THE NUTRITION OF THE RAT[1]

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(Received for publication March 17, 1938)

Though both ferrous and ferric salts of iron have been used in the treatment of nutritional anemia of the rat no direct evidence is available as to the relative efficiency of these two forms of iron under adequately controlled conditions. This is surprising in view of the experiments of Lintzel ('33) demonstrating that iron is absorbed in the rat in the ferrous form and the work of Reimann and Fritsch ('31) and Witts ('36) which indicates that ferric chloride is less potent than ferrous chloride or ferrous sulphate in the treatment of certain cases of hypochromic anemia in man.

It is the purpose of this paper to describe experiments designed to test the relative utilization of these forms of iron when given as supplements to a milk diet and to an iron-low solid diet. These experiments form part of a general study being made in this laboratory of the factors which govern iron absorption and utilization.

MILK DIET

Experimental

Five male and five female rats were selected from three litters rendered highly anemic by the method of Elvehjem and Kemmerer ('31). These were separately caged and

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] Service Fellow of the Commonwealth Fund of New York.

divided into two groups kept as even as possible with respect to weight and hemoglobin levels and each containing three males and two females.

Treatment of the two groups throughout the 5-week experimental period was as follows: group 1 received whole milk plus 0.05 mg. Cu plus 0.3 mg. ferrous iron daily. The iron was given as a solution of ferrous ammonium sulphate made up fresh each week and maintained in the reduced form by the addition of a few crystals of sodium hydrosulphite. Group 2 received milk from the same source and the same level of copper plus 0.3 mg. of ferric iron daily as a solution of ferric ammonium sulphate. It will be noted that this level of supplementary feeding represents a slightly sub-optimal level of iron intake, thus preventing the masking of any differences in the effectiveness of the two forms of iron due to too high an intake.

Complete consumption of the mineral supplements was assured by feeding them in 10 cc. of milk to each rat each morning. The main milk supply was not given until the afternoon by which time the supplements had been fully consumed. For the first 2 weeks of the experiment the total intake of each rat in each group was restricted to 35 cc. milk daily. Thereafter the amount was gradually raised to 45 cc. daily. In this way any differences due to a variable intake of the basal diet were entirely avoided.

Results

Weight increases. The individual weight increases are given in table 1. The results are closely similar in the two groups and call for little comment. The mean rate of increase over the whole 5 weeks is close to 2 gm. per rat per day which is quite normal when mineralized 'winter' milk is used.

Hemoglobin levels. The individual levels of hemoglobin per 100 cc. blood are given in table 2. The gains throughout the experimental period are very similar in each group. However, it should be noted that the females do not show any greater gains in hemoglobin over the males in the first 2 weeks

TABLE 1

Live weights of rats. Milk diet

	5-4-37	5-17-37	5-22-37	5-27-37	6-2-37	6-8-37
	Group 1. Ferrous iron supplement					
	gm.	gm.	gm.	gm.	gm.	gm.
7791 ♂	60	68	79	89	99	102
7802 ♂	51	67	78	92	108	111
7803 ♂	58	69	78	95	115	121
7806 ♀	40	55	51	65	86	94
7811 ♀	65	82	94	99	101	94
Mean	55	68	76	88	102	106
	Group 2. Ferric iron supplement					
7801 ♂	45	61	68	82	90	100
7815 ♂	67	77	88	95	120	124
7805 ♂	47	63	77	86	110	115
7812 ♀	64	71	73	87	104	111
7813 ♀	48	62	69	86	97	103
Mean	54	67	75	87	104	111

TABLE 2

Hemoglobin levels. Milk diet. Grams per 100 cc. blood

	5-11-37	5-17-37	5-22-37	5-27-37	6-2-37	6-9-37
	Group 1. Ferrous iron supplement					
7791 ♂	4.45	8.42	11.10	10.20	10.81	9.64
7802 ♂	4.19	7.17	10.90	10.36	10.71	11.38
7803 ♂	4.58	7.45	10.36	11.29	8.78	10.44
7806 ♀	3.61	8.11	9.87	10.11	10.90	10.62
7811 ♀	3.90	7.19	8.96	11.48	11.59	12.77
Mean	4.15	7.67	10.24	10.37	10.56	10.97
	Group 2. Ferric iron supplement					
7801 ♂	4.78	9.02	11.48	10.71	10.62	11.71
7815 ♂	3.50	7.72	8.90	10.71	10.20	10.28
7805 ♂	4.02	7.37	10.28	11.29	10.71	10.62
7812 ♀	3.95	8.00	9.16	9.79	11.10	10.03
7813 ♀	3.65	7.17	10.36	9.57	9.95	11.00
Mean	3.98	7.86	10.03	10.42	10.52	10.75

as obtained in the experiments of Smith and Otis ('37). This lends support to the contention of Mitchell and Hamilton ('37) that the higher milk consumption of the males is the principal factor involved in this sex difference. In the present experiments the milk consumption of the males and females was, as indicated earlier, kept equal.

Iron storage in the livers. Since the liver is the principal storage organ of the body for iron it would be expected that liver iron determinations would give some indication of the relative extent of the absorption of iron above that utilized in hemoglobin building. In this way some of the confusion between absorbed iron and utilized iron which had occurred in much of the work on human anemias would be avoided. The recent work of Brock and Hunter ('37) and of Fowler and Barer ('37) show clearly that the poor utilization of some forms of iron used in the treatment of certain of the hypochromic anemias of man cannot be due to poor absorption, to which it was formerly attributed, since these forms of iron are absorbed and retained in the body in considerable quantities.

Accordingly at the completion of the 5-week period the rats were killed by decapitation, bled and their livers analyzed for total iron. The results are given in table 3.

Considerable variation is apparent in each group and considerable overlapping exists in the two groups. It is evident however that there is a tendency for the rats fed ferric iron to store more in their livers than those fed ferrous iron. Too much importance cannot be attached to this difference in view of the range of values obtained. In fact statistical treatment of the data shows the observed difference has rather less than a 20:1 chance of being real.

SOLID DIET

It is known from the work of Robinson and Duncan ('31) that a high lactose diet such as whole milk lowers the pH of the intestinal tract of the rat. This would tend to favor the absorption of iron through increasing the solubility. It

might also tend to minimize any superiority of ferrous over ferric salts since, as shown by Halvorsen and Starkey ('27) with pure solutions, a greater concentration of ferrous than ferric ions occur in such solutions at a pH of over 5. It was essential therefore to test the relative ability of the rat to utilize ferric and ferrous iron when given a solid lactose-free diet.

TABLE 5

Iron contents of livers. Milk diet

RAT. NO.	DRY WEIGHT LIVER	TOTAL Fe	MILLIGRAM Fe PER GRAM DRY WEIGHT
Ferrous iron group			
	gm.	mg.	
7791 ♂	1.38	0.27	0.20
7802 ♂	1.34	0.39	0.29
7803 ♂	1.62	0.28	0.18
7806 ♀	1.40	0.42	0.30
7811 ♀	1.44	0.49	0.34
Mean	1.44	0.37	0.26
Ferric iron group			
7801 ♂	1.30	0.58	0.44
7815 ♂	1.53	0.66	0.42
7805 ♂	1.39	0.48	0.34
7812 ♀	1.61	0.28	0.18
7813 ♀	1.21	0.65	0.53
Mean	1.41	0.53	0.38

Experimental

After some preliminary trials the following iron-low ration was developed and found to be satisfactory:

	%
White corn	12
Sucrose	57
Egg albumin	10
Purified casein	8
Butterfat	7.5
Liver extract powder	1.5
Salts	4

The salts consisted of 50% $CaCO_3$, 25% NaCl and 25% K_2HPO_4 plus sufficient copper to bring the intake up to 0.1 mg.

Cu per day. Additional vitamin B_1 was given as the pure hydrochloride and vitamins A and D as percomorph oil.

The total iron content of different batches of this ration ranged from 0.027 to 0.030 mg. Fe per gram and the 'available' iron, as determined by the dipyridyl method, from 0.012 to 0.014 mg. Fe per gram. That is, the iron of this ration was only 44 to 46% as available as inorganic iron fed at the same level.

Seven pairs of litter mates were selected from four litters and rendered highly anemic on a whole milk diet as before. They were then placed on the above ration, the paired feeding technic being used so that the daily food consumption of each rat was the same as its mate. In addition one rat of each pair received a daily supplement of 0.25 mg. Fe in the ferric form and the other member of each pair 0.25 mg. Fe in the ferrous form. Two rats were kept as controls and given no supplement.

The ration was quite well consumed and quickly rose from an average of 4 gm. to almost 9 gm. per rat per day. The two control rats never consumed more than 6 gm. per day each.

Results

Weight increases. The mean weight increases of the seven rats given ferrous iron and of the seven rats given ferric iron, together with that of the controls, are given in table 4. Though the rates of gain are much lower than that of the milk-fed rats in the previous experiment, particularly in the first 2 weeks, no differences are evident between the ferrous and the ferric iron groups.

Hemoglobin levels. The mean hemoglobin levels in grams per 100 cc. of blood are given in table 5.

The iron deficiency of the basal diet is evident by the negligible gains in hemoglobin levels made throughout by the control rats. The rats receiving the iron supplements, on the other hand, made fairly good gains during this period. There is an apparent small advantage from the ferrous iron supplement, as compared to the ferric, during the first 2 weeks but

the difference is not statistically significant. During the rest of the period the levels are exceedingly close in the two groups.

TABLE 4

Live weights of rats. Solid diet

	1937					
	11/3	11/10	11/17	11/24	12/1	12/8
Ferrous iron supplement						
	gm.	gm.	gm.	gm.	gm.	gm.
Mean of seven rats	63	65	74	83	92	104
Range	50–75	51–81	59–92	63–97	68–106	75–125
Ferric iron supplement						
Mean of seven rats	64	65	75	82	94	104
Range	47–74	50–79	58–91	62–96	70–107	78–122
Basal diet only						
Mean of two rats	62	60	64	68	76	80

TABLE 5

Hemoglobin levels. Solid diet. Grams per 100 cc. blood

	1937					
	11/3	11/10	11/17	11/24	12/1	12/8
Ferrous iron supplement						
Mean of seven rats	3.86	6.88	7.95	9.77	10.01	10.11
Range	2.78–5.06	6.20–7.45	7.35–9.16	8.42–10.90	8.54–11.59	9.36–11.59
Ferric iron supplement						
Mean of seven rats	3.99	6.23	7.69	9.03	9.84	10.36
Range	2.50–5.45	4.41–7.20	6.75–8.90	8.20–9.95	8.90–10.71	9.36–11.10
Basal diet only						
Mean of two rats	3.68	4.00	4.29	5.15	4.74	4.96

Iron storage in the liver. As before the animals were killed at the end of the experimental period, bled and their livers analyzed for total iron. The results are given in table 6.

The individual results are quite variable in each group, though not to the same extent as found in the previous experiment using milk. The levels of storage are also generally lower than that of the milk fed rats, though no difference between those fed ferrous and ferric iron is evident.

TABLE 6

Iron content of livers. Solid diet

	DRY WEIGHT LIVER	TOTAL Fe	Fe PER GRAM DRY WEIGHT
Ferrous iron supplement			
Mean of seven rats	1.25	0.25	0.20
Range	1.06–1.60	0.16–0.33	0.15–0.31
Ferric iron supplement			
Mean of seven rats	1.36	0.28	0.21
Range	1.22–1.52	0.16–0.36	0.13–0.32

DISCUSSION

The results show clearly that ferrous and ferric salts of iron at limited intakes are utilized equally effectively by the anemic rat. If, as claimed by Lintzel ('33), iron is absorbed from the intestine only in the ferrous form then it is evident that the anemic rat, either on a milk diet or on a lactose-free solid diet, has no difficulty in reducing ingested ferric iron to the ferrous form.

A point of interest arising from the experiments is the much more efficient utilization of iron on the milk diet than on the solid diet. This is particularly noticeable in the first 2 weeks of the two experimental periods. During this time the rats on the supplemented milk diet made an average increase in the hemoglobin level of 6.08 gm. per 100 cc. blood while increasing their live weights an average of 21 gm. per rat. On the solid diet, on the other hand, the corresponding increase in hemoglobin level is only 3.40 gm. per 100 cc. and the live weight only 11 gm. per rat. The 'available' iron intake per rat during this time was 0.30 gm. Fe per day in

the case of the solid diet and 0.38 gm. Fe in the case of the milk diet, if 100% 'availability' of the iron in milk be assumed. If the iron in milk is less than 100% 'available' then the difference in iron utilization on the two diets would become even more striking.

The reason for the poorer iron utilization on the solid diet is not at present understood though it is difficult to resist the conclusion that it is associated, at least in part, with the higher intestinal acidity induced by the milk diet. A study of the effect on iron utilization of alkalization of the diet is at present being undertaken in this laboratory.

SUMMARY

Experiments to test the relative efficiency of ferrous and ferric iron at limited intakes in inducing growth, hemoglobin regeneration and storage of iron in the liver in the anemic rat, on a milk diet and on a lactose-free solid diet, are described.

The two forms of iron were found to be equally effective for these purposes on both types of diet, though the iron was much better utilized on the milk than on the solid diet at similar levels of intake.

ACKNOWLEDGMENT

Acknowledgment is made to Profs. E. B. Hart and C. A. Elvehjem for advice and criticism and for placing the facilities of the Laboratory of Agricultural Chemistry at my disposal.

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